

# Eukaryotic Linear Motifs on the ELM database

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## Keywords

Linear motifs, Bioinformatics, Protein-Protein Interaction, Molecular switches, Cell regulation

## Significance statement

*instructions: Provide a 120-word-maximum statement about the significance of the protocols/topic described in your manuscript. This should be understandable to undergraduate-educated scientists outside their field of specialty. The goal is to explain the relevance of the work in broad context to a broad readership. It will be used in promotion of the article following publication.*

## Abstract

*instructions: brief overview, no references, max 150 words*

The Eukaryotic Linear Motif (ELM) resource (<http://elm.eu.org>) is a manually curated database of short linear motifs (SLiMs). This protocol explains how to best use this resource and explains how to access the database content (both manual and scripted access), how to interpret the output, and how to predict novel putative motifs in any given protein sequence.

## Introduction

The activity and function of a protein is tightly regulated by its cellular environment. To interact with their surroundings, proteins use various types of binding modules that each display distinct binding properties (Wright and Dyson (1999)). One prominent type of binding module consists of short linear motifs (SLiMs) (Diella (2008)). These compact binding sites are generally located in intrinsically disordered regions (IDR) of the proteome and commonly bind to surface of a globular domain in a protein (Davey et al. (2012)). SLiMs mediate different types of interactions that regulate protein functionality, and hence are important regulators of the dynamic processes involved in cell signalling (Van Roey et al. (2012)) (Van Roey et al. (2014)) (Figure 1). The number of SLiM instances in the human proteome is currently suggested to be over one million (Tompa et al. (2014)). Identifying SLiMs and elucidating their functionality is an essential step in understanding cell regulation. The Eukaryotic Linear Motif (ELM) resource contributes to this process by providing the necessary tools to researchers working on motifs. It consists of a database

and a prediction tool. The database provides a categorised repository of experimentally validated linear motif classes and instances that were manually annotated from the literature. The ELM prediction tool in turn relies on annotated data, both from the ELM database and other resources, to accurately analyse unknown sequences for candidate motifs and assist researchers in selecting the most plausible ones for experimental validation and discard likely false positive hits, saving them valuable time and assets (Dinkel et al. (2012)). The following protocols will guide users through the different ELM applications, explaining how to browse the curated data available in ELM, how to analyse a protein sequence for putative motifs, and how to interpret these data and avoid common pitfalls in SLiM discovery.

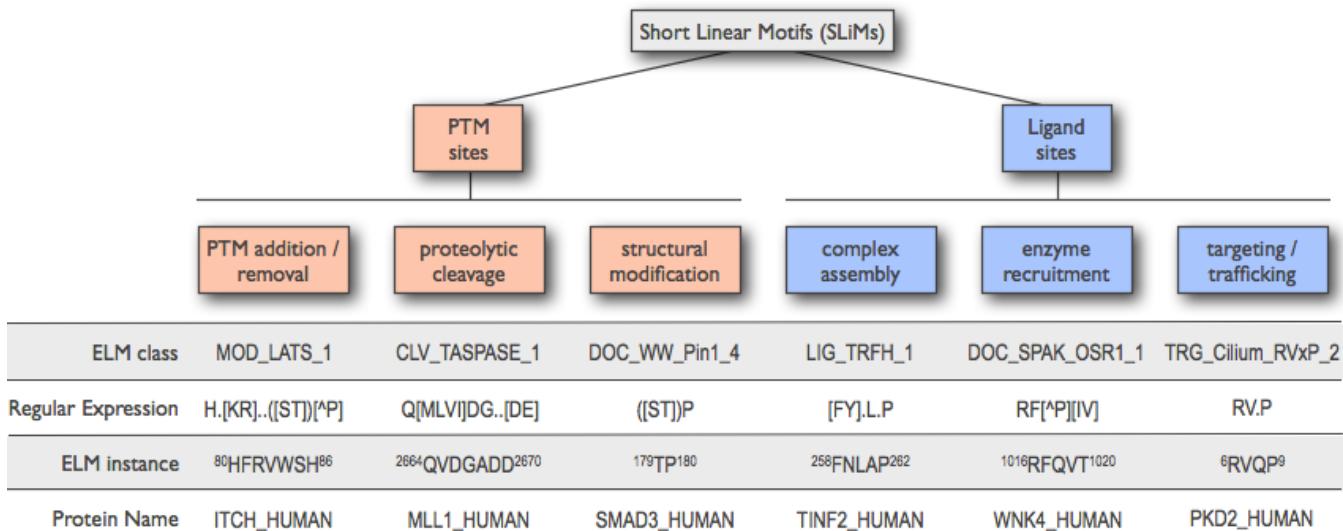


Figure 1: **Figure functional\_classification\_of\_SLiMs** For each ELM class, the functional category to which it belongs is indicated by a three-letter prefix. Each ELM class is defined by a regular expression. Peptide sequences in proteins that match the regular expression of a specific ELM class and that were experimentally validated to be functional motifs are captured as ELM instances of that class. Degrons are a specific subtype of enzyme-recruiting docking motifs (see text for a detailed description).

## Protocol 1 (Basic): Explore the content of the ELM DB

The core of the ELM database is a repository of manually annotated motifs and instances. As of December 2016, ELM contains over 260 motif classes categorized into 6 different types: DOC (docking), LIG (Ligand binding), DEG (degradation), CLV (cleavage), MOD (post translational modifications), and TRG (targeting/anchoring) motifs (Figure functional\_classification\_of\_SLiMs). These motifs are derived from various types of experiments reported in literature. Each manually annotated motif also has a set of bona fide instances (occurrences) of this motif. Currently, there are over 3000 annotated instances annotated from over 2500 publications. The motif classes and motif instances have been uploaded by a large group of annotators from around the globe. The complete catalogue of manually curated data can be searched, browsed and explored on the ELM website

Figure 2: **Figure TP53-BP2-1** The ELM database overview page (<http://elm.eu.org/search.db>).

## Protocol 1.1 Database content overview

1. Go to <http://elm.eu.org> and click on the tab “ELM DB” to explore the content of the different types of data about experimentally validated ELMs that were manually curated from the literature (Fig. 2). This page contains a brief summary of the database content, as well as the number of links to third-party databases. The table gives an overview of the type and amount of information stored in the database. Each line contains at least one link which will take you to the corresponding contents page (eg. “ELM instances”).

## Protocol 1.2 Browsing motif classes and annotated instances

2. Click on the sub-menu “ELM classes” in “ELM DB” to see the page with all of the ELM classes (Fig. 3). For each class, the following information is provided: ELM identifier, short description, regular expression, number of instances annotated for each class, and number of structures available. For details on each class, click on the ELM identifier; to get a list of annotated instances for an individual

Figure 3: Figure TP53-BP2-2 The list of all motifs in the ELM database.

class, click on the number of instances.

*Use the search bar at the top of the page to filter for certain motif classes. For example, typing “MAPK” and hitting submit will perform a full-text search on all motif classes in the ELM database containing the term “MAPK”. The green buttons on the left can also be used to filter this table. For example, toggling the “DOC” button will remove all “DOC” classes from the table (and clicking it again will bring them back). Lastly, the yellow tsv link can be used to export all motif classes as a “tab separated values” file.*

3. Search the table for the term “DOC\_CYCLIN\_1” and click on its link to navigate to the page with details about the “DOC\_CYCLIN\_1” motif class (Fig. 4). This page contains a description of the functional site class (a Cyclin recognition site), and a short description of the ELM and its regular expression, as well as a probability score, the taxonomic distribution of the motif and which domain (if any) is responsible for the interaction.

*The probability score is the probability that the regular expression represents a random selection*

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«DOC\_CKS1\_1» »DOC\_GSK3\_Axin\_1»

## DOC\_CYCLIN\_1

**Accession:** ELM**E00010**

**Functional site class:** Cyclin recognition site

**Functional site description:** Functional site that interacts with cyclins, and thereby increases the specificity of phosphorylation by cyclin/CDK complexes.

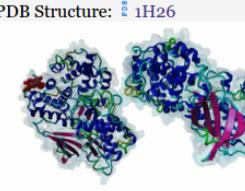
**ELM Description:** Substrate recognition site that interacts with cyclin and thereby increases phosphorylation by cyclin/cdk complexes. Predicted proteins should have a CDK phosphorylation site (**MOD\_CDK\_1**). Also used by cyclin/cdk inhibitors.

**Pattern:** [RK] . L. {0,1} [FYLIVMP]

**Pattern Probability:** 0.0053239

**Present in taxon:** Eukaryota

**Interaction Domain:** Cyclin\_N (PF00134) Cyclin, N-terminal domain (Stoichiometry: 1:1)

**PDB Structure:** 1H26 

See 24 Instances for DOC\_CYCLIN\_1

**Abstract**  
The cyclin recognition site (alias Cy or RxL motif) is found in a wide range of cyclin/CDK interacting proteins (Takeda, 2001). The presence of this motif in CDK substrates substantially increases the level of phosphorylation at ([ST])Px[KR] motifs (**MOD\_CDK\_1**). Example proteins are the retinoblastoma protein, E2F 1-3 and p53. CDK phosphorylation mainly occurs in the nucleus but there also is some evidence for cytoplasmic function. For example, the cytoplasmic SRC and TAU proteins are known cyclin/CDK targets. The motif is recognised by a conserved region in the cyclin protein and binds in a similar manner as the p21Kip cyclin inhibitor (1J8U).

4 selected references: Show

5 GO-Terms: Show

Figure 4: **Figure TP53-BP1- 4** The motif details page for “DOC\_CYCLIN\_1”. This page contains all of the manual annotation details for the DOC\_CYCLIN\_1 motif, the biological background summarized from the scientific literature including links to the primary literature and to external resources (Pubmed (Coordinators (2017)), GeneOntology (Consortium (2017)), PDB (Berman et al. (2002)) and more).

of amino acids (similar to an information content score). A lower score indicates that the motif pattern is more difficult to find by chance in a random sequence.

- Further down the “DOC\_CYCLIN\_1” page (Fig. 4) to view more details about the manually annotated data and instances in the database (to the text box starting with the “Abstract”). The “abstract” contains a more detailed description of the motif annotation. Click on the “Show” button next to the “selected references” header for a list of publications relevant to this motif. Click on “Show” next to “GO terms” for a complete list of all GO terms annotated for this motif.
- Scroll further down the “DOC\_CYCLIN\_1” page to view the “Instances” header (Fig. 5) This table contains the list of all annotated instances in the database of this motif. This includes the protein identifier, the start and end positions of the instance, the specific sequence matching the

■ 24 Instances for DOC\_CYCLIN\_1  
(click table headers for sorting; Notes column: ⚡=Number of Switches, 🌐=Number of Interactions)

Acc., Gene-, Name	Start	End	Subsequence	Logic	#Ev.	Organism	Notes
P04637 TP53_P53_HUMAN	381	385	GQSTS <small>RHKKLMFKTEGPDS</small> D	TP	4	Homo sapiens (Human)	1H26
P46527 CDKN1B_CDKN1B_HUMAN	30	33	EHPKPSACRNL <small>F</small> GPVDHEEL	TP	5	Homo sapiens (Human)	1H27 1JSU
P38936 CDKN1A_CDKN1A_HUMAN	19	22	NPCGSKACRRLF <small>G</small> PVDSSEQL	TP	4	Homo sapiens (Human)	1 1
P06789 E1_VE1 HPV18	127	130	NSGQKKAKRRLFTISDGYG	TP	3	Human papillomavirus type 18	1
Q99741 CDC6_CDC6_HUMAN	94	98	HSHTLKG <small>RRLV</small> FDNQLTIKS	TP	2	Homo sapiens (Human)	2CCH
Q14207 NPAT_NPAT_HUMAN	1062	1066	AAKPCHR <small>RVLCF</small> DSTTAPVA	TP	1	Homo sapiens (Human)	
P39880 CUX1_CUX1_HUMAN	1301	1305	NYRSRIR <small>REL</small> FIEEIQAGSQ	TP	1	Homo sapiens (Human)	
P38826 ORC6_ORC6_YEAST	178	182	ESPSITR <small>RKLAF</small> EEDEDDE	TP	1	Saccharomyces cerevisiae (Baker's yeast)	
Q9WTQ5 Akap12_AKA12_MOUSE	501	504	IKVQGSPLKKL <small>FSSGL</small> KKL	TP	1	Mus musculus (House mouse)	1
Q00716 E2F3_E2F3_HUMAN	134	138	GGGPPAKRRL <small>E</small> GESGHQYL	TP	1	Homo sapiens (Human)	
Q14209 E2F2_E2F2_HUMAN	87	91	AGRLPAKRKL <small>DLE</small> GIgrpVV	TP	1	Homo sapiens (Human)	
Q01094 E2F1_E2F1_HUMAN	90	94	LGRPPVKRRLD <small>L</small> ETDHQYLA	TP	3	Homo sapiens (Human)	1H24
P50445 rux_RUX_DROME	248	251	PTARRCVR <small>RTL</small> FTEENTQKE	TP	1	Drosophila melanogaster (Fruit fly)	
Q13352 ITGB3BP_CENPR_HUMAN	5	9	MPVKRSLKLDGLLEENSFDP	TP	1	Homo sapiens (Human)	
Q8UWJ8 CDH1-A_Q8UWJ8_CHICK	394	398	KLTGHSYRVLYLA <small>MSPDGEA</small>	FP	1	Gallus gallus (Chicken)	
P07305 H1F0_H10_HUMAN	73	76	DSQIKLSIKRKL <small>V</small> TGVLKQT	FP	1	Homo sapiens (Human)	
P49918 CDKN1C_CDKN1C_HUMAN	31	34	VLVRTSACRSLF <small>G</small> PVDHEEL	TP	1	Homo sapiens (Human)	

Figure 5: **Figure ???** The instances annotated for DOC\_CYCLIN\_1.

regular expression and the logic of the instance. The “# Ev.” indicates the number of experimental evidences associated with the annotation (see section XXX below). Organism is the species in which the protein is found. Lastly the “Notes” column contains links to any “interactions” or “switches” present in the database, as well as links to PDB if this structure exists in PDB.

- Click on the sub-menu “ELM instances” in “ELM DB” to go to the page which lists all of the instances in the database (Fig. 6). This table contains a list of all instances in the database.

*Use the search filters at the top of the page to limit the results by a full text search, by instance logic, or organisms. Similar to the ELM classes page (previous step) these results can be filtered by motif class using the green toggle filters on the left hand side. Lastly, the yellow buttons at the top of the page can be used to download the instances in the following formats: gff, pir, fasta or tsv.*

- Type “p53\_human” in the search box to search for ELM Instances in this protein. Find the row for the ELM class “DOC\_CYCLIN\_1” and click on the startposition “381” to go to the instance details

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Search ELM Instances

Full-Text Search (use "\*" to get all instances)

Filter by instance Logic

Filter by organism

submit Reset

export 100 instances as: gff pir fasta tsv

CLV	DEG	DOC	LIG	MOD	TRG	ELM identifier	Acc., Gene-, Name	Start	End	Subsequence	Logic	#Ev.	Organism	Notes
						DOC_MAPK_HePTP_8	P08018 PBS2 PBS2_YEAST	217	234	SLSARRGKLPPGGMISLKH	U	1	Saccharomyces cer...	1
						DOC_MAPK_HePTP_8	P35236 PTPN7 PTN7_HUMAN	38	50	HVR <del>LQERRGSNVNALMDVRS</del>	TP	6	Homo sapiens (Human)	2GPH 1
						DOC_MAPK_HePTP_8	P15822 HIVEP1 ZEP1_HUMAN	1422	1437	P <del>LERRGPLVROTSLNAP</del>	TP	1	Homo sapiens (Human)	2
						DOC_MAPK_HePTP_8	Q15256 PTPRR PTPR_HUMAN	333	345	P <del>IQLQERRGSNVSLTLDMSS</del>	TP	3	Homo sapiens (Human)	1
						DOC_MAPK_HePTP_8	P54829 PTPN5 PTN5_HUMAN	239	251	SMG <del>LQERRGSNVSLTLDMCT</del>	TP	5	Homo sapiens (Human)	3
						DOC_MAPK_HePTP_8	Q62132 Ptprr PTPR_MOUSE	332	344	P <del>IQLQERRGSNVSLTLDMSS</del>	TP	3	Mus musculus (House mouse)	2
						DOC_MAPK_HePTP_8	P06784 STE7 STE7_YEAST	7	19	RKT <del>LQRNLKGQLNLNLPDV</del>	TP	9	Saccharomyces cer... (Baker's yeast)	2B9H 3
						DOC_MAPK_HePTP_8	P38590 MSG5 MSG5_YEAST	26	38	PR <del>SQNRTKKNLSDTAALH</del>	TP	3	Saccharomyces cer... (Baker's yeast)	2B9I 1
						DOC_MAPK_HePTP_8	Q6PJF5 RHBDF2 RHDf2_HUMAN	19	31	SSR <del>LQSRKPPNLSTTIPPE</del>	TP	1	Homo sapiens (Human)	1
						DOC_MAPK_HePTP_8	Q96CC6 RHBDF1 RHDF1_HUMAN	12	24	TSS <del>LQRKKPPWLKDIPSAV</del>	TP	3	Homo sapiens (Human)	1

Figure 6: **Figure TP53-BP2-3** The list of all instances in the ELM database.

page of this instance. The top part of the page contains details about the instance and the protein it was identified in.

8. Scroll down to the “Instance Evidence” header to view details on the experimental evidence used to annotate this instance. This table also contains the “evidence class”, and descriptions of the methods used from PSI-MI ([Kerrien et al. \(2007\)](#)) as well as the Literature references in which the experiments were published.

(*Here we should explain what “evidence class”, “biosource”, “Logic”, “Reliability” and “Notes” actually mean*).

### Protocol 1.3 Switches, motif-mediated pathways and other external resources.

9. Scroll further down to the header “Pathways” to view pathway information. This is a list of all of the pathways in which the protein p53 is known to be involved (according to KEGG). Click on a pathway to see the localization of p53 in the corresponding KEGG pathway.

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**DOC CYCLIN\_1**

■ Instance

Accession	Acc. Gene-, Name	Start	End	Subsequence	Logic	PDB	Organism	Length
ELMI000051	P04637 TP53 P53_HUMAN	381	385	GQSTSRH <b>KKLMF</b> KTEGPDS	TP	1H26	Homo sapiens (Human)	393

■ Instance evidence

Evidence class	PSMI	Method	BioSource	PubMed	Logic	Reliability	Notes
experimental	MI:0405	competition binding	in vitro	Luciani,2000	support	certain	InteractionDetection
experimental	MI:0074	mutation analysis	in vivo/in vitro	Luciani,2000	support	certain	FeatureDetection
experimental	MI:0065	isothermal titration calorimetry	in vitro	Lowe,2002	support	certain	InteractionDetection
experimental	MI:0114	x-ray crystallography	in vitro	Lowe,2002	support	certain	InteractionDetection FeatureDetection

■ Pathways

The sequence P04637 is implicated in the following 35 Pathways: (color codes: This sequence=red, interacting sequence=orange)

- Amyotrophic lateral sclerosis (ALS)
- Apoptosis
- Basal cell carcinoma
- Bladder cancer
- Cell cycle
- Central carbon metabolism in cancer
- Chronic myeloid leukemia
- Colorectal cancer
- Endometrial cancer
- Epstein Barr virus infection
- Glioma
- HTLV I infection
- Hepatitis B
- Hepatitis C
- Herpes simplex infection

Figure 7: **Figure TP53-BP1- 5** The instance details page for the “DOC\_CYCLIN\_1” instance annotated for protein P53\_HUMAN with start/end position “381-385”. This page also contains links to many external databases including Uniprot (Consortium (2015)), PDB (Berman et al. (2002)), NCBI taxonomy, Pubmed (Coordinators (2017)), and KEGG Pathways (Kanehisa et al. (2016)), as well as the PSI-MI controlled vocabulary (Kerrien et al. (2007)).

10. Repeat the previous search by clicking on the sub-menu “ELM instances” in “ELM DB” and type “p53\_human” in the search box. This time, try to find the ELM instance “DOC\_WW\_Pin1\_4” motif with the start/end position “30-35” (You can sort the table by clicking on the header lines eg. on “Start” to sort by startposition ). Click on the start/endposition or the subsequence which will take you to the details page as shown in figure 8. This page is similar to that described for the P53 instance “DOC\_CYCLIN\_1” (Fig. 7); additionally, for this instance there is information available about its interaction partner and a molecular switch which is mediated by this motif instance.
11. Scroll down to the “Interactions” header to view information about this instance’s interactions (Fig. 8). This instance interacts with PIN1\_Human via the “WW” domain (PFAM identifier PF00397; found on position 7–37 in PIN1\_Human). If available, binding affinities are also shown here. Inter-

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**DOC WW Pin1\_4**

**Instance**

Accession	Acc. Gene-, Name	Start	End	Subsequence	Logic	PDB	Organism	Length
ELMI001957	P04637 TP53 P53_HUMAN	30	35	WKLLPEN <b>NVLSPL</b> PSQAMDD	TP	---	Homo sapiens (Human)	393

**Instance evidence**

Evidence class	PSMI	Method	BioSource	PubMed	Logic	Reliability	Notes
experimental	MI:0059	gst pull down	in vivo/in vitro	Wulf,2002	support	certain	InteractionDetection
experimental	MI:0074	mutation analysis	in vivo/in vitro	Wulf,2002	support	certain	FeatureDetection

**Interactions**

Uniprot Id	Domain family	Domain Start	Domain End	Affinity Min/Max ( $\mu$ Mol)	Notes
(Q13526) PIN1_HUMAN	PF00397 (WW) WW domain	7	37		[mitab] [xml]

**Switches**

This ELM instance is part of the following 1 switching mechanism annotated at the switches.ELM resource:

- SWTI000037:

Phosphorylation of S33 in the Pin1-binding motif of Cellular tumor antigen p53 (TP53) induces binding to the Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1) protein.

Figure 8: **Figure TP53-BP1-6** The instance details page for the “DOC\_WW\_Pin1\_4” instance found in P53 with start/end position “30-35”.

action data is made available in Mitab and xml format (Kerrien et al. (2007)).

12. Scroll further down to the “Switches” section for a brief overview of the switches details of this instance obtained form “switches.ELM” (Van Roey et al. (2013a)) (Fig. 8). This particular instance is involved in the switch phosphorylating P53. Clicking on the diagram will open an external link to the “switches.ELM” website.
13. Click on the sub-menu “ELM methods” in “ELM DB” to see a list of all experimental methods which have been used to identify motifs and instances (Fig 9). This table shows the internal method identifier in the first column, a link to the corresponding entry in the PSI-MI database (Kerrien et al. (2007)), and the method name as annotated by the PSI-MI controlled vocabulary, as well as the type of experiment (in vitro/in vivo). Clicking on the link in the “instances” column will list all instances annotated using that method.

*The filter bar on the top page can be used to filter the list of methods. The tsv link creates a*

The screenshot shows the ELM (Eukaryotic Linear Motif) database interface. At the top, there is a navigation bar with links to "ELM Home", "ELM Prediction", "ELM DB", "ELM Candidates", "ELM Information", "ELM downloads", and "Help". The main content area has a title "112 different methods used in ELM annotation". Below this, a table lists various experimental methods, each with a unique ID (e.g., 98 MI:0004, 9 MI:0005), their PSIMI ID, name, biosource, interaction type, number of instances, and notes. A search bar at the top of the table allows filtering by search term, and an "export as: tsv" link is available.

ID	PSIMI ID	Method	Biosource	Interaction	#Instances	Notes
98 MI:0004		Affinity Chromatography Technology	in vivo/in vitro	association	36	InteractionDetection
9 MI:0005		Alanine Scanning	in vivo/in vitro/in silico		327	FeatureDetection
37 MI:0257		Antisense RNA	in vivo		3	InteractionDetection
67 MI:0007		Anti Tag Coimmunoprecipitation	in vivo	association	114	InteractionDetection
277 MI:0010		Beta Galactosidase Complementation			2	InteractionDetection
309 MI:0809		Bimolecular Fluorescence Complementation			8	InteractionDetection
156 MI:0969		Biolayer Interferometry			1	InteractionDetection
327 MI:0968		Biosensor			1	InteractionDetection
458 MI:2163		By Homology	in silico	association	10	ParticipantIdentification
203 MI:0225		Chromatin Immunoprecipitation Array			2	InteractionDetection
104 MI:0402		Chromatin Immunoprecipitation Assay	in vivo	association	2	InteractionDetection
137 MI:0091		Chromatography Technology	in vitro	physical association	16	InteractionDetection
18 MI:0016		Circular Dichroism	in vitro	association	19	InteractionDetection
65 MI:0017		Classical Fluorescence Spectroscopy	in vitro	association	119	InteractionDetection
405 MI:0990		Cleavage Assay			10	InteractionDetection
129 MI:0194		Cleavage Reaction	in vivo/in vitro		50	InteractionDetection
23 MI:0019		Coimmunoprecipitation	in vivo/in vitro	association	563	InteractionDetection
16 MI:0403		Colocalization	in vitro		152	
146 MI:0807		Comigration In Gel Electrophoresis in vitro			7	InteractionDetection
123 MI:0404		Comigration In Non Denaturing Gel Electrophoresis	in vivo	association	4	InteractionDetection
132 MI:0808		Comigration In Sds Page	in vitro		5	InteractionDetection
12 MI:0405		Competition Binding	in vitro	physical	105	InteractionDetection

Figure 9: **Figure TP53-BP2-4** The list of all experimental methods used in the ELM database.

downloadable file in “tab separated values” format.

- Click on the sub-menu “ELM pdb structures” in “ELM DB” to see a list of all macromolecular structures in the ELM database (Fig. 10). Structures annotated in ELM ideally (but not always) show both interaction partners, motif and domain. This page also contains links to RCSB (Berman et al. (2002)), the individual instance and the motif class of that instance.

*The filter bar on the top page can be used to filter the list of structures shown . The tsv link creates a downloadable file in “tab separated values” format. The tsv file contains the PDB id, uniprot name, and ELM class.*

- Click on the sub-menu “ELM binding domains” in “ELM DB” to see a complete list of all the interaction domains in ELM (Fig. 11). This table shows the ELM classes which have been annotated with a corresponding interaction domain. This table shows the ELM class, a link to the Pfam (Finn et al. (2016)) / SMART (Letunic et al. (2015)) / InterPro (Finn et al. (2017)) domain, as well as the name of the interacting domain followed by a brief description.

PDB_ID	Title	ELM instance	ELM class
<a href="#">2FOP</a>	The crystal strucure of the n-terminal domain of hausp/usp7 complexed with mdm2 peptide 147-150	MDM2_HUMAN	DOC_USP7_MATH_1
<a href="#">2FOO</a>	The crystal strucure of the n-terminal domain of hausp/usp7 complexed with p53 peptide 359-362	P53_HUMAN	DOC_USP7_MATH_1
<a href="#">2G2L</a>	Crystal structure of the second pdz domain of sap97 in complex with a glut-a c-terminal peptide	GRIA1_RAT	LIG_PDZ_Class_1
<a href="#">2G30</a>	Beta appendage of ap2 complexed with arh peptide	ARH_HUMAN	TRG_AP2beta_CARGO_1
<a href="#">2GBQ</a>	Solution nmr structure of the grb2 n-terminal sh3 domain complexed with a ten-residue peptide derived from sos direct refinement against noes, j-couplings, and 1h and 13c chemical shifts, 15 structures	SOS1_MOUSE	LIG_SH3_3
<a href="#">2GPH</a>	Docking motif interactions in the map kinase erk2	PTN7_HUMAN	DOC_MAPK_HePTP_8
<a href="#">2GPO</a>	Estrogen related receptor-gamma ligand binding domain complexed with a synthetic peptide from rip140	NRIP1_HUMAN	LIG_NRBOX
<a href="#">2GTH</a>	Crystal structure of the wildtype mhv coronavirus non-structural protein nsp15	R1AB_CVMA5	LIG_Rb_LxCxE_1
<a href="#">2HE2</a>	Crystal structure of the 3rd pdz domain of human discs large homologue 2, dlg2	AT2B4_HUMAN	LIG_PDZ_Class_1
<a href="#">2HE4</a>	The crystal structure of the second pdz domain of human nherf-2 (slc9a3r2) interacting with a mode 1 pdz binding motif	DHRS2_HUMAN	LIG_PDZ_Class_1
<a href="#">2HG0</a>	Structure of the west nile virus envelope glycoprotein	Q3I0Y8_WNV	MOD_N-GLC_1
<a href="#">2HKQ</a>	Crystal structure of the c-terminal domain of human eb1 in complex with the cap-gly domain of human dynactin-1 (p150-glued)	MARE1_HUMAN	LIG_CAP-Gly_1
<a href="#">2I04</a>	X-ray crystal structure of magi-1 pdz1 bound to the c-terminal peptide of hpv18 e6	VE6 HPV18	LIG_PDZ_Class_1
<a href="#">2I01</a>	X-ray crystal structure of sap97 pdz3 bound to the c-terminal peptide of hpv18 e6	VE6 HPV18	LIG_PDZ_Class_1
<a href="#">2I0L</a>	X-ray crystal structure of sap97 pdz2 bound to the c-terminal peptide of hpv18 e6.	VE6 HPV18	LIG_PDZ_Class_1
<a href="#">2I1N</a>	Crystal structure of the 1st pdz domain of human dlg3	AT2B4_HUMAN	LIG_PDZ_Class_1
<a href="#">2I3S</a>	Bub3 complex with bub1 glebs motif	BUB1_YEAST	LIG_GLEBS_BUB3_1
<a href="#">2I3T</a>	Bub3 complex with mad3 (bubr1) glebs motif	MAD3_YEAST	LIG_GLEBS_BUB3_1
<a href="#">2OVQ</a>	Structure of the skp1-fbw7-cyclinedegc complex	CCNE1_HUMAN	DEG_SCF_FBW7_1
<a href="#">2P1K</a>	Crystal structure of dynein light chain lc8 in complex with a peptide derived from swallow	SWA_DROME	LIG_Dynein_DLC8_1
<a href="#">2P1L</a>	Structure of the bcl-xl:beclin 1 complex	BECN1_HUMAN	LIG_BH_BH3_1

Figure 10: **Figure TP53-BP2-5** The list of all known structures in PDB also in ELM.

*The filter bar on the top page can be used to filter the list of interactions shown. The tsv link creates a downloadable file in “tab separated values” format.*

#### Protocol 1.4 Links to external resources

- Click on the sub-menu “ELM switches” in “ELM DB” to see a complete list of all the switches in ELM (Fig. 12). This table shows the motif class, contains a link to Uniprot, and the start and stop positions of the motif mediating the switch. The last two columns have links to switches.ELM, and a brief description of the switch also taken from switches.ELM (Van Roey et al. (2013a)).

*The filter bar on the top page can be used to quickly filter the list of interactions shown.*

#### Protocol 1.5 Exploring KEGG pathways from ELM

- Click on the sub-menu “ELM pathways” in “ELM DB” to see a list of all pathways contained in ELM (Fig. 13). Pathways are from the “Kyoto Encyclopedia of Genes and Genomes” (KEGG

The screenshot shows the ELM (Eukaryotic Linear Motif) database interface. At the top, there's a navigation bar with links to 'ELM Home', 'ELM Prediction', 'ELM DB', 'ELM Candidates', 'ELM Information', 'ELM downloads', and 'Help'. The main content area has a title 'The Eukaryotic Linear Motif resource for Functional Sites in Proteins' and a search bar labeled 'Search ELM Database'. Below this, a table titled '290 interaction domains annotated in ELM' is displayed. The table has columns for 'ELM identifier', 'Interaction Domain Id', 'Interaction Domain Name', and 'Interaction Domain Description'. The table lists various entries such as CLV\_NRD\_NRD\_1, CLV\_PCSK\_FUR\_1, CLV\_PCSK\_PC1ET2\_1, etc., each with its corresponding PF ID and domain name. A 'Filter this table' input field and a 'searchTerm' input field are located above the table. An 'export as:' button with a 'tsv' option is at the top right of the table.

ELM identifier	Interaction Domain Id	Interaction Domain Name	Interaction Domain Description
CLV_NRD_NRD_1	PF00675	Peptidase_M16	Insulinase (Peptidase family M16)
CLV_PCSK_FUR_1	PF00082	Peptidase_S8	Subtilase family
CLV_PCSK_PC1ET2_1	PF00082	Peptidase_S8	Subtilase family
CLV_PCSK_PCT7_1	PF00082	Peptidase_S8	Subtilase family
CLV_PCSK_SKI1_1	PF00082	Peptidase_S8	Subtilase family
CLV_TASPASE1	PF01112	Asparaginase_2	Asparaginase
old_LIG_14-3-3_1	PF00244	14-3-3	14-3-3 protein
old_LIG_14-3-3_2	PF00244	14-3-3	14-3-3 protein
old_LIG_14-3-3_3	PF00244	14-3-3	14-3-3 protein
LIG_AP_GAE_1	PF02883	Alpha_adaptinC2	Adaptin C-terminal domain
LIG_AP2alpha_1	PF02296	Alpha_adaptin_C	Alpha adaptin AP2, C-terminal domain
LIG_AP2alpha_2	PF02296	Alpha_adaptin_C	Alpha adaptin AP2, C-terminal domain
DEG_APCC_DBOX_1	PF00400	WD40	WD domain, G-beta repeat
DEG_APCC_KENBOX_2	PF00400	WD40	WD domain, G-beta repeat
LIG_BIR_II_1	PF00653	BIR	Inhibitor of Apoptosis domain
LIG_BIR_III_1	PF00653	BIR	Inhibitor of Apoptosis domain
LIG_BIR_III_2	PF00653	BIR	Inhibitor of Apoptosis domain
LIG_BIR_III_3	PF00653	BIR	Inhibitor of Apoptosis domain
LIG_BIR_III_4	PF00653	BIR	Inhibitor of Apoptosis domain
LG_BRCB_BRCB_1	PF00701	BRCB	BRCB domain

Figure 11: **Figure TP53-BP2-6** A list of all interactions annotated in the database.

Kanehisa et al. (2016)) database mapped to ELM instances. Click on a species (for example “Homo sapiens”) for a complete list of all Human pathways which have a protein annotated in ELM, and links to the pathways on KEGG.

18. On the “ELM pathways” page (Fig. 14) click on the link “gallus gallus” to navigate to the page containing all pathways annotated for chicken. This page contains links to all KEGG pathways for the taxon *gallus gallus* with annotated instances in the ELM database.
19. One the page with chicken pathways (Fig. 15) click on “Adherens junction” to the KEGG entry for this pathway, with proteins color overlay corresponding to ELM classes (see the color legend right side of figure 15).

## Protocol 1.6 Infections and Diseases

20. Click on the sub-menu “ELM virus instances” in “ELM DB” to see a list of all instances in ELM that have been annotated as being abused by viruses (Fig. 16). The columns are identical to those

The Eukaryotic Linear Motif resource for Functional Sites in Proteins

Search ELM Database

ELM Home ELM Prediction ELM DB ELM Candidates ELM Information ELM downloads Help

### ELM Switches

Filter this table [searchTerm]

ELM class	Sequence Id	Start/Stop	Switch Id	Description
LIG_SH2_STAT5	P043561	161-164	SWTI000001	Phosphorylation of Y161 in the SH2-binding motif of <a href="#">Linker for activation of T-cells family member 1 (LAT)</a> induces binding to the <a href="#">1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-1 (PLCG1)</a> protein.
DOC_AGCK_PIF_1	P31749	469-474	SWTI000002	Phosphorylation of S473 in the PIF motif of <a href="#">RAC-alpha serine/threonine-protein kinase (AKT)</a> by <a href="#">Serine/threonine-protein kinase mTOR (MTOR)</a> (as part of mTORC2 complex) induces intramolecular interaction with the PIF-binding pocket, resulting in cistivivation of <a href="#">RAC-alpha serine/threonine-protein kinase (AKT)</a> . Dephosphorylation of the PIF motif by PHLPP1/2 (PHLPP1 for Akt2/3 and PHLPP2 for Akt1/3) results in reduced Akt activity, probably by disrupting the interaction with the Akt PIF pocket and thus cistivivation.
DOC_AGCK_PIF_1	P05771-2	656-661	SWTI000003	Dephosphorylation of the PIF motif by PHLPP1/2 results in reduced stability and increased degradation of PKC. This is countered by autophosphorylation of the PIF motif, but mTORC2 might also contribute.
DOC_WW_Pin1_4	Q12800	326-331	SWTI000004	Phosphorylation of T329 in the Pin1-binding motif of <a href="#">Alpha-globin transcription factor CP2 (TFCP2)</a> induces binding to <a href="#">Peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1)</a> , which isomerizes the peptide bonds at the nearby-phosphorylated SP motifs (S291 and S309) to the trans configuration, thereby facilitating their dephosphorylation, which is required for the transcriptional activity of <a href="#">Alpha-globin transcription factor CP2 (TFCP2)</a> .
LIG_PLK	P30307	129-131	SWTI000005	Phosphorylation of T130 in the PLK-docking motif of <a href="#">M-phase inducer phosphatase 3 (CDC25C)</a> by <a href="#">Cyclin-dependent kinase 1 (CDK1)-Cyclin AB subfamily</a> generates a recruitment site for <a href="#">Serine/threonine-protein kinase PLK1 (PLK1)</a> , which then phosphorylates <a href="#">M-phase inducer phosphatase 3 (CDC25C)</a> . This results in inactivation of the NES of <a href="#">M-phase inducer phosphatase 3 (CDC25C)</a> , thereby promoting its nuclear localization.
LIG_PLK	P30305	49-51	SWTI000006	Phosphorylation of S50 in the PLK-docking motif of <a href="#">M-phase inducer phosphatase 2 (CDC25B)</a> by <a href="#">Cyclin-dependent kinase 1 (CDK1)-Cyclin AB subfamily</a> generates a recruitment site for <a href="#">Serine/threonine-protein kinase PLK1 (PLK1)</a> , which then phosphorylates and activates <a href="#">M-phase inducer phosphatase 2 (CDC25B)</a> .
LIG_FHA_1	P64897	19-25	SWTI000007	Phosphorylation of T21 in the FHA-binding motif of <a href="#">Uncharacterized protein Rv1827/MT1875 (Rv1827)</a> by <a href="#">Probable serine/threonine-protein kinase pknG (pknG)</a> results in auto-inhibition due to an intramolecular interaction with the FHA domain. As a result, phosphorylation-independent interactions of the FHA domain with metabolic enzymes, which regulate the catalytic activity of these enzymes, are blocked (See also <a href="#">switch details</a> ).
LIG_14-3-3_3	P30307	213-218	SWTI000008	Phosphorylation of S216 in a 14-3-3-binding motif of <a href="#">M-phase inducer phosphatase 3 (CDC25C)</a> by <a href="#">Serine/threonine-protein kinase Chk1 (CHEK1)</a> induces binding to <a href="#">14-3-3 protein beta/alpha (YWHAB)</a> , which negatively regulates <a href="#">M-phase inducer</a> .

Figure 12: **Figure TP53-BP2-7** A list of all switches annotated in ELM.

listed in section XXX step YYY (Figure ZZZZ).

The green buttons on the left can be used to filter this table by motif class. Click on the yellow links on the top right of the page to download the (complete) table in gff, pir, fasta or tsv format. (See section XXX for a description of these formats.)

21. Click on the sub-menu “ELM diseases” in “ELM DB” to see a list of all motif classes that have been annotated with a disease (Fig. 17) Disease information is taken from the OMIM database.

This table also includes the diseases found under the “ELM pathogenic abuse” menu in “ELM DB”. (right?)

The screenshot shows the ELM (Eukaryotic Linear Motif) website. At the top, there is a navigation bar with links to "ELM Home", "ELM Prediction", "ELM DB", "ELM Candidates", "ELM Information", "ELM downloads", and "Help". The main content area has a title "Pathways linked from ELM instances". Below the title is a search bar with the placeholder "Please select a taxon or enter any search term:" followed by a "submit" button. A list of pathways is displayed as a bulleted menu, including: Arabidopsis thaliana (4), Ashbya gossypii ATCC 10895 (1), Bos taurus (65), Caenorhabditis elegans (5), Candida albicans SC5314 (1), Canis lupus familiaris (3), Danio rerio (6), Drosophila melanogaster (12), Equus caballus (2), Gallus gallus (16), Homo sapiens (231), Mus musculus (167), Oryctolagus cuniculus (29), Plasmodium falciparum 3D7 (1), Rattus norvegicus (139), Saccharomyces cerevisiae (26), Saccharomyces cerevisiae S288c (10), Schizosaccharomyces pombe (11), Schizosaccharomyces pombe 972h- (6), Solanum lycopersicum (1), Strongylocentrotus purpuratus (1), Sus scrofa (30), Vibrio cholerae (2), Xenopus laevis (23), and ALL (792). At the bottom of the page, there is a note about citation (PMID: 26615199), a link to the Software License Agreement, and an email address for feedback: feedback@elm.eu.org.

Figure 13: **Figure TP53-BP2-9** A list of all Pathways from KEGG with proteins in ELM.

## Protocol 2 (Alternate) General Search Box

A general search text box is available to explore the manually curated information in the ELM DB. This is a “Full-text” search which is performed on selected columns of the database therefore some attention (better term?) should be applied when evaluating the retrieved results.

1. Example 1: perform a search using the keyword ‘p53’. The results are retrieved in the following section: ELM instances (xx matches), ELM Switch (xx matches), and ELM Candidate classes (xx matches). One will find proteins such as CDH1\_YEAST (because of its accession P53197) which may or may not be what one wants.
2. Example 2: perform a search using the gene id TP53 or the UniProt Acc P04637. The results are retrieved in the following section: ELM instances (xx matches), ELM Switch (xx matches). The retrieved hits are less, but more specific compared with the search with ‘53’. However, there are no matches in ELM Candidate classes tough some content is related to the p53 protein.

The screenshot shows the ELM (Eukaryotic Linear Motif) database interface. At the top, there is a navigation bar with links to "elm Home", "elm Prediction", "elm DB", "elm Candidates", "elm Information", "elm downloads", and "Help". The main content area has a title "Pathways linked from ELM instances". Below the title is a table listing various biological pathways and their associated ELM instances and sequences. To the right of the table is a sidebar with information about KEGG database redirects and a color legend for pathway classes.

TAXON	Pathway entry	Pathway name	# Instances	# Sequences
	gga04520	Adherens junction	2	2
	gga04144	Endocytosis	2	2
	gga04012	ErbB signaling pathway	4	2
	gga04510	Focal adhesion	9	6
	gga04540	Gap junction	1	1
	gga04912	GnRH signaling pathway	4	2
	gga05168	Herpes simplex infection	3	1
	gga05164	Influenza A	3	1
	gga04010	MAPK signaling pathway	3	1
	gga04810	Regulation of actin cytoskeleton	5	4
	gga05132	Salmonella infection	3	1
	gga04530	Tight junction	1	1
	gga04620	Toll like receptor signaling pathway	3	1
	gga04270	Vascular smooth muscle contraction	2	1
	gga04370	VEGF signaling pathway	2	2
	gga04310	Wnt signaling pathway	4	2

Figure 14: **Figure TP53-BP2-10** A list of all pathways in *Gallus Gallus*

## Protocol 3 (Basic) Predicting ELMs in sequences

One of the most useful (and used) features in ELM is the ability to detect motifs in proteins and sequences. Given a protein's amino acid sequence, the “ELM Predictions” pipeline searches for occurrences of each motif class using regular expressions, apply a set of filters to help judging results, and to visualize resulting set of putative motifs.

In this protocol we will be viewing the manually annotated data of a typical protein, using p53 (Uniprot ID: P53\_HUMAN/P04637) as an example. We will cover how to find the manually annotated motifs and instances, and how to find the motif instances, the references used to annotate each instance, the experimental protocols used, and additional information including relationships to biological pathways (such as KEGG [Kanehisa et al. \(2016\)](#)), diseases (from OMIM [McKusick \(2007\)](#)) and molecular switches (in switches.ELM [Van Roey et al. \(2013a\)](#)).

### Protocol 3.1 Necessary Resources

#### Protocol 3.1.1 Software & Hardware

A modern browser such as Firefox, Chrome, or Safari. ELM is best viewed on a laptop or desktop computer, although tablets and smartphones will also work.

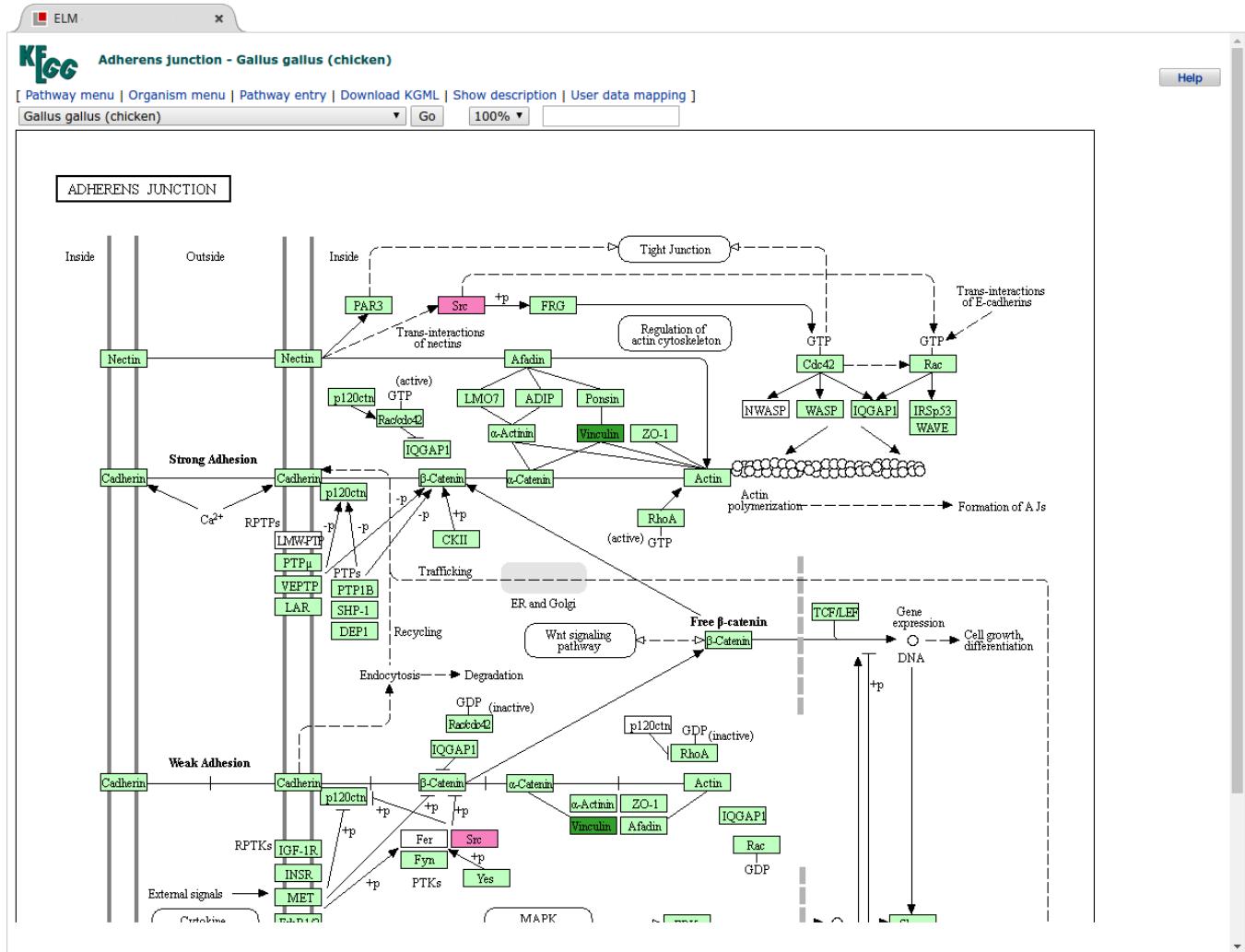


Figure 15: **Figure TP53-BP2-11** A list of all annotated pathways for taxon *gallus gallus*

### Protocol 3.2 Predicting ELM instances using data from ELM database

1. Open a browser, and navigate to the ELM homepage: <http://elm.eu.org>. Enter the Uniprot ID “P53\_HUMAN” in the search field labelled “Enter a uniprot identifier or accession number”. The page should autocomplete/suggest the protein “P53\_HUMAN / P04637 (Homo sapiens)”. Click on this entry to confirm that we want to search for SLiM data for this protein. Click on “Submit” to view the motif instance data for p53. (22)

*The autocompletion mechanism queries uniprot.org for protein identifier; if it succeeds, then additional information from uniprot will be used to pre-populate the filter boxes. In this example, P53\_HUMAN is recognized as a Human protein, and so “Homo sapiens” is automatically filled in the “Taxonomic Context” field. Also, P53 has been annotated (by Uniprot) to be localized to nucleus, cytosol, endoplasmic reticulum and mitochondrion, so these are also automatically applied as search criteria. The motif cutoff of “100” is a sufficiently high (lenient) threshold to allow all other detected motifs to be shown.*

The screenshot shows the ELM (Eukaryotic Linear Motif) web interface. At the top, there's a navigation bar with links to ELM Home, ELM Prediction, ELM DB, ELM Candidates, ELM Information, ELM downloads, and Help. Below the navigation is a search bar labeled "Search ELM Database". The main content area is titled "Browse Viral ELM Instances". A table lists 242 instances, with an option to export them in gff, pir, fasta, or tsv formats. The table has columns for ELM identifier, Accession, Gene-, Name, Start, End, Subsequence, Logic, #Ev., Organism, and Notes. A sidebar on the left contains a vertical list of motif categories: CLV, DEG, DOC, LIG, MOD, and TRG.

ELM identifier	Acc., Gene-, Name	Start	End	Subsequence	Logic	#Ev.	Organism	Notes
CLV_PCSK_FUR_1	P056861 env ENV_FFV	124	128	GNTSSSSR <del>R</del> RDIQYHKLPV	TP	4	Feline foamy virus	1 1
CLV_PCSK_FUR_1	P03383 env ENV_HTLV2	305	309	PVPPPAT <del>R</del> RRRAVPIAVWLV	TP	3	Human T-lymphotropic virus	1 1
CLV_PCSK_FUR_1	P03375 env ENV_HV1B1	508	512	AKRRVVQ <del>R</del> EKKRAVGIGALFL	TP	3	Human immunodeficiency virus type I	1 1
CLV_PCSK_FUR_1	P03420 F FUS_HRSVA	133	137	NVTLSKK <del>R</del> KRRLFGLFLGVG	TP	3	Human respiratory syncytial virus	1 1
CLV_PCSK_FUR_1	P03188 gB GB_EBVB9	429	433	TPAAVL <del>R</del> RRRDAGNATTPV	TP	1	Human herpesvirus... (Epstein-Barr virus (strain B95-8))	
CLV_PCSK_FUR_1	P27909 POLG_DEN1B	202	206	SQTGEHR <del>R</del> KRSVALAPHG	TP	3	Dengue virus 1	1 1
CLV_PCSK_FUR_1	P11223 S SPIKE_IBVB	534	538	KITNGTR <del>R</del> FRRSITENVANC	TP	2	Avian infectious ...	
CLV_PCSK_FUR_1	P11223 S SPIKE_IBVB	687	691	LLTNPSS <del>R</del> KRSLIEDLLLFT	TP	2	Avian infectious ...	
CLV_PCSK_FUR_1	Q05320 GP VGP_EBOZM	498	502	GLITGGRR <del>T</del> RREAIIVNAQPK	TP	4	Ebola virus - May...	1 1
CLV_PCSK_FUR_1	P03107 L2 VL2 HPV16	9	13	RHKRSAA <del>R</del> TKRASATQLYKT	TP	1	Human papillomavirus	1 1
CLV_PCSK_FUR_1	P60170 GP VSGP_EBOZM	321	325	EPKTSVV <del>R</del> VRELLPTQPT	TP	3	Ebola virus - May...	
DEG_APCC_KENBOX_2	P03116 E1 VE1_BPV1	27	31	TEAECE <del>S</del> D <del>K</del> E <del>N</del> E <del>P</del> GAGVEL	TP	1	Bovine papillomavirus	
DEG_SCF_FBW7_2	P03070 Large T antigen LT_SV40	699	705	ICRGFTCFKK <del>P</del> PTPPP <del>P</del> PET	TP	3	Simian virus 40	1 1

Figure 16: **Figure TP53-BP2-11** A Table of the ELM instance abused by viruses

- Select the search criteria (optional). It is possible to limit the results by “cell compartment”, “taxonomic context” or by changing the “motif probability cutoff”. To restrict the search to include SLiMs that are active in certain cellular compartments, select one or more from the list (use the “control” key to select more than one option). It is also possible to select a “taxonomic context” to restrict the search to SLiMs from certain species. Start typing a species name in the “taxonomic context” input field to get an auto-completed list of species to select from. Additionally, a “Motif probability cutoff” can be used to only retain ELM classes whose pattern probability is below the given value. For the current protocol, leave all of these at their default values: “not specified”, “100” and no “taxonomic context”

TODO: Repeat search using stringent filters (homo sapiens, nucleus, 0.01)

### Protocol 3.3 Interpreting the prediction results: Graphical Summary

- Click “submit” to start the searching for motifs. You will be brought to an intermediate page indicating that your results are being processed, and you should be redirected to the final results page

Figure 17: **Figure TP53-BP2-8** A list of all diseases in ELM.

within a minute. You can bookmark this page: The results are stored for a week.

*The Results are summarized in the first figure on the results page (see figure 23). The graphical summary shows the results generated by the ELM prediction pipeline, combined with additional filters and information from external resources. The visualization should help you interpreting the results and to assess whether or not a motif is present in a sequence, as well as how likely it is to be functional based on its structural context and evolutionary conservation. Motif instances which are manually annotated in the database appear as red (TP) or yellow (FP) ovals in the graphic. Blue/gray squares represent predicted motif occurrences.*

4. The first row contains phosphorylation sites as retrieved from Phospho.ELM (Dinkel et al. (2011)), and whether the phosphorylated amino acid is a serine, threonine or tyrosine. Phospho.ELM is a database of manually annotated phosphorylation sites obtained from scientific publications from low and high-throughput experiments. You can follow the link to Phospho.ELM by clicking on the phosphorylation site in the image to get more information on individual phosphorylation sites.

The screenshot shows the ELM (Eukaryotic Linear Motif) website interface. At the top, there is a navigation bar with links to 'ELM Home', 'ELM Prediction', 'ELM DB', 'ELM Candidates', 'ELM Information', 'ELM downloads', and a user account section for 'admin'. Below the navigation bar, a search bar displays the query 'p53'. The main content area is titled 'Your search for p53 resulted in 0 ELM classes, 31 ELM Instances, 5 ELM candidate classes, and 44 ELM Switches:'. A section titled 'ELM instances' contains a table listing 31 ELM instances found for the query. The table has columns for Identifier, Sequence, Start, Stop, Logic, Taxon, and Info.

Identifier	Sequence	Start	Stop	Logic	Taxon	Info
DEG_APCC_DBOX_1	P53350 PLK1 PLK1_HUMAN	336	344	TP	Homo sapiens (Human)	
DEG_APCC_TPR_1	P53197 CDH1 CDH1_YEAST	564	566	TP	Saccharomyces cer...	
DEG_MDM2_SWIB_1	P04637 TP53 P53_HUMAN	19	26	TP	Homo sapiens (Human)	1YCR
DOC_CYCLIN_1	P04637 TP53 P53_HUMAN	381	385	TP	Homo sapiens (Human)	1H26
DOC_MAPK_gen_1	P53355 DAPK1 DAPK1_HUMAN	1385	1393	FP	Homo sapiens (Human)	
DOC_PP2B_PxIxI_1	P53968 CRZ1 CRZ1_YEAST	330	336	TP	Saccharomyces cer... (Baker's yeast)	
DOC_USP7_MATH_1	P04637 TP53 P53_HUMAN	364	368	TP	Homo sapiens (Human)	2FOJ
DOC_USP7_MATH_1	P04637 TP53 P53_HUMAN	359	363	TP	Homo sapiens (Human)	2F1X 2FOO
DOC_WW_Pin1_4	P04637 TP53 P53_HUMAN	312	317	TP	Homo sapiens (Human)	1A
DOC_WW_Pin1_4	P04637 TP53 P53_HUMAN	30	35	TP	Homo sapiens (Human)	1A
DOC_WW_Pin1_4	P04637 TP53 P53_HUMAN	78	83	TP	Homo sapiens (Human)	1A
Fungi and Amoebozoa.">LIG_APCC_Cbox_2	P53197 CDH1 CDH1_YEAST	55	61	TP	Saccharomyces cer...	
LIG_CaM_IQ_9	P53141 MLC1 MLC1_YEAST	84	102	TP	Saccharomyces cer... (Baker's yeast)	
LIG_CID_NIM_1	P53632 PAP2 PAP2_YEAST	574	583	TP	Saccharomyces cer...	2MOW
	DE3200_XAP1800					

Figure 18: **Figure TP53-AP1-1**

*Phosphorylation sites are only available when the search is performed with a protein accession (eg. not with FASTA sequence alone) in step XXX and there is relevant information annotated in the Phospho.ELM database. Phosphorylation sites are relevant to interpret ELM motif predictions when the predicted motif requires to be phosphorylated (as in several docking and ligand binding motifs) and naturally, for the prediction of phosphorylation motifs.*

5. The second row shows SMART and Pfam domains detected by the SMART database (Schultz et al. (1998), Letunic et al. (2015), Schultz et al. (1998)). Hover the mouse over these domains to see their names and exact start and end positions.

*In order to be functional SLiMs need to be accessible, and therefore they are usually not found within globular domains and structured regions (Davey et al. (2012)). Any SLiMs detected by the ELM prediction pipeline are less likely to be functional, and are indicated with a red background (see also the “structural filter” described in step XXX).*

6. The third row shows globular and disordered regions in the sequence as predicted by GlobPlot

Diagram	Switch	Description
CDH1 	SWTI000687	The KEN-box motif of <i>APC/C-CDH1 modulator 1 (ACM1)</i> binds to the substrate recruitment site of <i>APC/C activator protein CDH1 (CDH1)</i> , the substrate recognition subunit of the Anaphase Promoting Complex/Cyclosome (APC/C), and thereby blocks recruitment, and subsequent targeting for proteasomal degradation, of the Cdh1 substrate <i>G2/mitotic-specific cyclin-2 (CLB2)</i> . Degradation of <i>G2/mitotic-specific cyclin-2 (CLB2)</i> is required for mitotic exit and maintenance of the G1 phase of the cell cycle and is allowed by Cdc20-dependent degradation of <i>APC/C-CDH1 modulator 1 (ACM1)</i> in anaphase.
CDH1 	SWTI000688	The KEN-box motif of <i>APC/C-CDH1 modulator 1 (ACM1)</i> binds to the substrate recruitment site of <i>APC/C activator protein CDH1 (CDH1)</i> , the substrate recognition subunit of the Anaphase Promoting Complex/Cyclosome (APC/C), and thereby blocks recruitment, and subsequent targeting for proteasomal degradation, of the Cdh1 substrate <i>Kinesin-like protein CIN8 (CIN8)</i> . Degradation of <i>Kinesin-like protein CIN8 (CIN8)</i> is required for mitotic exit and maintenance of the G1 phase of the cell cycle and is allowed by Cdc20-dependent degradation of <i>APC/C-CDH1 modulator 1 (ACM1)</i> in anaphase.
CDH1 	SWTI000689	The KEN-box motif of <i>APC/C-CDH1 modulator 1 (ACM1)</i> binds to the substrate recruitment site of <i>APC/C activator protein CDH1 (CDH1)</i> , the substrate recognition subunit of the Anaphase Promoting Complex/Cyclosome (APC/C), and thereby blocks recruitment, and subsequent targeting for proteasomal degradation, of the Cdh1 substrate <i>Probable serine/threonine-protein kinase HSL1 (HSL1)</i> . Degradation of <i>Probable serine/threonine-protein kinase HSL1 (HSL1)</i> is required for mitotic exit and maintenance of the G1 phase of the cell cycle and is allowed by Cdc20-dependent degradation of <i>APC/C-CDH1 modulator 1 (ACM1)</i> in anaphase.
CDH1 	SWTI000689	The KEN-box motif of <i>APC/C-CDH1 modulator 1 (ACM1)</i> binds to the substrate recruitment site of <i>APC/C activator protein CDH1 (CDH1)</i> , the substrate recognition subunit of the Anaphase Promoting Complex/Cyclosome (APC/C), and thereby blocks recruitment, and subsequent targeting for proteasomal degradation, of the Cdh1 substrate <i>Probable serine/threonine-protein kinase HSL1 (HSL1)</i> . Degradation of <i>Probable serine/threonine-protein kinase HSL1 (HSL1)</i> is required for mitotic exit and maintenance of the G1 phase of the cell cycle and is allowed by Cdc20-dependent degradation of <i>APC/C-CDH1 modulator 1 (ACM1)</i> in anaphase.
CDH1 	SWTI000688	The KEN-box motif of <i>APC/C-CDH1 modulator 1 (ACM1)</i> binds to the substrate recruitment site of <i>APC/C activator protein CDH1 (CDH1)</i> , the substrate recognition subunit of the Anaphase Promoting Complex/Cyclosome (APC/C), and thereby blocks recruitment, and subsequent targeting for proteasomal degradation, of the Cdh1 substrate <i>Kinesin-like protein CIN8 (CIN8)</i> . Degradation of <i>Kinesin-like protein CIN8 (CIN8)</i> is required for mitotic exit and maintenance of the G1 phase of the cell cycle and is allowed by Cdc20-dependent degradation of <i>APC/C-CDH1 modulator 1 (ACM1)</i> in anaphase.
CDH1		The KEN-box motif of <i>APC/C-CDH1 modulator 1 (ACM1)</i> binds to the substrate recruitment site of <i>APC/C activator protein CDH1 (CDH1)</i> , the substrate recognition subunit of the Anaphase Promoting Complex/Cyclosome (APC/C), and thereby blocks

Figure 19: **Figure TP53-AP1-2**

(Linding et al. (2003)). The fourth and fifth row contains results from IUPred (Dosztányi et al. (2005)), another predictor of disordered protein regions. Protein segments with an IUPred score above 0.5 considered to be disordered.

*SLIMs are typically only functional when found in intrinsically disordered regions. Any motif occurrence detected by the ELM prediction pipeline that falls within disordered regions are more likely to be functional.*

7. The 5th row contains information on secondary structure. The secondary structure is predicted using a pipeline mapping motif occurrence onto high quality reference domain structures (Via et al. (2009)). Check the graphical representation, if the output of the secondary structure filter and the disorder predictors agree with respect to which parts of the sequence are considered structured and which disordered.
8. The remainder of the figure (below “secondary structure” output) displays predicted and annotated motif instances, overlayed by the structural context from rows 2 and 3 (SMART domains and Glob-

**ELM**

XPO1

PLK1

XPO1

SWTI000186

Phosphorylation of S198 in the NES of *M-phase inducer phosphatase 3 (CDC25C)* by *Serine/threonine-protein kinase PLK1 (PLK1)* inhibits binding to *Exportin-1 (XPO1)*, thus promoting nuclear localization of *M-phase inducer phosphatase 3 (CDC25C)*.

XPO1

Splicing event

XPO1

SWTI000518

Alternative splicing removes the nuclear export signal (NES) of *Cellular tumor antigen p53 (TP53)*, abrogating binding to *Exportin-1 (XPO1)* and export from the nucleus.

**ELM candidate classes**

Identifier	Regex	Reference	Description	Notes	Status	Info
LIG_CagA		<a href="#">24474782</a> , <a href="#">4IRV</a>	N-terminal domain of the Cytotoxin Associated Gene A (CagA) of Helicobacter pylori binds to a 20aa long helical motif in the Apoptosis-stimulation protein p53-2 (ASPP2).		<a href="#">first draft</a>	<a href="#">[Edit]</a> <a href="#">[Delete]</a>
LIG_KIX_CBP	[DEST][LMYI]..[LIF][LIV]	<a href="#">22474372</a> , <a href="#">2KWF</a> , <a href="#">16253272</a> , <a href="#">2AGH</a> , <a href="#">9413984</a> , <a href="#">1KDX</a> , <a href="#">19220000</a> , <a href="#">17467953</a>	hydrophobic motif found in transcription factors (FOXO3a, CREB, c-Myb, p53, TCF4...) that interacts with the KIX domain of CBP/p300 to recruit this transcription coactivator. Promiscuous as they might also bind to TAZ1 and TAZ2 domains of CBP/p300. For FOXO3a, phosphorylation of overlapping serine increases affinity.		<a href="#">first draft</a>	<a href="#">[Edit]</a> <a href="#">[Delete]</a>
LIG_R3IM	[DE][DE] [DE]EFE[DE]	<a href="#">18775730</a>	Motif of the DSS1 protein required for proteasome interaction and p53 protein degradation.		<a href="#">first draft</a>	<a href="#">[Edit]</a> <a href="#">[Delete]</a>
MOD_acetylation		<a href="#">10656693</a> , <a href="#">10607594</a> , <a href="#">9744860</a> , <a href="#">9774110</a> , <a href="#">9809067</a>	Acetylation targets in the nucleus beyond histone tails: p53, HMG I/Y, TCF, etc.		<a href="#">first draft</a>	<a href="#">[Edit]</a> <a href="#">[Delete]</a>
LIG_PH_Tfb1	[ILVF]..W[ILVF].[DE]	<a href="#">16793543</a> , <a href="#">2GS0</a>	Amphipathic helix motif in P53 that is recognised by the PH domain of the p62 subunit of TFIID. 3uM and phosphorheostatic binding (pS46 518nM, pT55 457nM and pS46pT55 97nM).		<a href="#">undergoing annotation</a>	<a href="#">[Edit]</a> <a href="#">[Delete]</a>

Please cite: ELM 2016-data update and new functionality of the eukaryotic linear motif resource. (PMID: [26615199](#)) [feedback@elm.eu.org](#)

ELM data can be downloaded & distributed for non-commercial use according to the [ELM Software License Agreement](#)

Figure 20: **Figure TP53-AP1-3**

Plot). A blue square indicates a single motif occurrence, intensity of the color indicates the conservation of this sequence in homologous proteins. Boxes in gray are motif occurrences which have been filtered out by the “structure filter”. Boxes that are blue & gray are neutral (eg. residing in structural context, but the secondary structure detected a loop region). If the sequence is already present in the ELM database, any motif instances that have already been annotated are shown as ovals. Lastly, any motifs detected, which are annotated to be functional in homologous sequences, are shown as red/blue rectangles.

#### TODO: EXPLAIN / SHOW ANNOTATED INSTANCES

*In the case that not enough homologous sequences were detected to build an alignment, no conservation score can be calculated. Therefore all of the motif occurrences will be shown in a uniform shade of blue.*

9. Place the cursor over the blue box for motif occurrence “MOD\_PLK” at position 6-12. This motif is in a disordered region, and has not been filtered out by the structural filter. However, its conservation

The Eukaryotic Linear Motif resource for Functional Sites in Proteins

Your search for p0463 resulted in 0 ELM classes, 14 ELM Instances, 0 ELM candidate classes, and 11 ELM Switches:

### ELM instances

Identifier	Sequence	Start	Stop	Logic	Taxon	Info
DEG_MDM2_SWIB_1	P04637 TP53 P53_HUMAN	19	26	TP	Homo sapiens (Human)	1YCR
DOC_CYCLIN_1	P04637 TP53 P53_HUMAN	381	385	TP	Homo sapiens (Human)	1H26
DOC_USP7_MATH_1	P04637 TP53 P53_HUMAN	359	363	TP	Homo sapiens (Human)	2F1X 2FOO
DOC_USP7_MATH_1	P04637 TP53 P53_HUMAN	364	368	TP	Homo sapiens (Human)	2FOJ
DOC_WW_Pin1_4	P04637 TP53 P53_HUMAN	78	83	TP	Homo sapiens (Human)	1A
DOC_WW_Pin1_4	P04637 TP53 P53_HUMAN	312	317	TP	Homo sapiens (Human)	1A
DOC_WW_Pin1_4	P04637 TP53 P53_HUMAN	30	35	TP	Homo sapiens (Human)	1A
MOD_CDK_SPxxK_3	P04637 TP53 P53_HUMAN	315	319	TP	Homo sapiens (Human)	
MOD_CK1_1	P04637 TP53 P53_HUMAN	15	21	TP	Homo sapiens (Human)	
MOD_GSK3_1	P04637 TP53 P53_HUMAN	30	37	TP	Homo sapiens (Human)	1A
MOD_PIKK_1	P04637 TP53 P53_HUMAN	12	18	TP	Homo sapiens (Human)	
MOD_SUMO_for_1	P04637 TP53 P53_HUMAN	385	388	TP	Homo sapiens (Human)	
TRG_NES_CRM1_1	P04637 TP53 P53_HUMAN	339	352	TP	Homo sapiens (Human)	1A
TRG_NLS_Bipartite_1	P04637 TP53 P53_HUMAN	305	323	TP	Homo sapiens (Human)	

Figure 21: **Figure TP53-AP1-4**

score is very low: 0.16, indicating it is not conserved in homologous proteins.

*The confidence score is based on how conserved the sequence is across a set of homologous proteins from other sequences. An full description of the method can be found in Chica et al. (2008).*

10. Mouse over a gray rectangle (indicating motifs which have been filtered out) to find out why this hit was filtered out. It shows scores for all of the individual criteria used by the secondary structure filter: The name of the domain, the *accessibility score*, *secondary structure score*, *combined total score*, and the associated *total score P-value* (Via et al. (2009)).
- TODO: INSERT/CHANGE FIGURE/NAME
11. Scroll down to below the results graphic to find additional information on the ELM Prediction pipeline's results (Fig. 24). The first section contains links to download or view the multiple sequence alignments of homologous proteins used to calculate the conservation score. Click on the link "Click here to enable the multiple sequence alignment viewer" to open the alignment in Jalview

ELM

## ELM Prediction

The **ELM prediction** tool scans user-submitted protein sequences for matches to the regular expressions defined in ELM. Distinction is made between matches that correspond to experimentally validated motif instances already curated in the ELM database and matches that correspond to putative motifs based on the sequence. Since SLiMs are short and degenerate, overprediction is likely and many putative SLiMs will be false positives. However, predictive power is improved by using additional filters based on contextual information, including taxonomy, cellular compartment, evolutionary conservation and structural features.

**Protein sequence**

Enter Uniprot identifier or accession number: (auto-completion)  
e.g. [EPN1\\_HUMAN](#), [P04637](#), [TAU\\_HUMAN](#), [\[RANDOM\]](#)  
[P53\\_HUMAN](#)

Or paste the sequence (Single letter code sequence only or FASTA format):  
>P53\_HUMAN  
>P53\_HUMAN  
MEEPQSDPSVEPPLSQETFSDLWKLPPENNVLSPLPSQAMDDILMLSPDDIEQWFTEDPGPDEAPRMPPEAAPVAPAPAAPTFA  
APAPAPSWPLSPVSPQRKYGGYGRFLHSGTAKSVCTYSPALNMFCQLAKTCFVQLWMDSTPPPGTRVRAIYKQS  
QHMTEVVRCCPHHERCSDSGLAPPQHLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEPVGSDCTTHYNMCMNSCMGGMNRR  
PILTIITLEDSSGNNLLGRNSFEVRCACPGDRRTEENLKKGEPHHELPGSTRKALPNNTSSPQPKKKPLDGEYFTLQI  
RGRRERFEMFREIMALELKDAQAGKEPGGSRAHSSHLSKKGQSTSRRHKLMFKTEGPDSD

**Cell compartment (one or several):**

- not specified
- extracellular
- nucleus
- cytosol
- peroxisome
- glycosome
- glyoxisome
- Golgi apparatus
- endoplasmic reticulum
- lysosome
- endosome
- plasma membrane
- mitochondrion

**Taxonomic Context**

Type in species name (auto-completion):  
[Homo sapiens](#)

**Motif Probability Cutoff:**

100

**Submit** **Reset Form**

**ELM DB**

The ELM relational database stores different types of data about experimentally validated SLiMs that are manually

peptide from ELM  
class LIG\_PTB\_Apo\_2

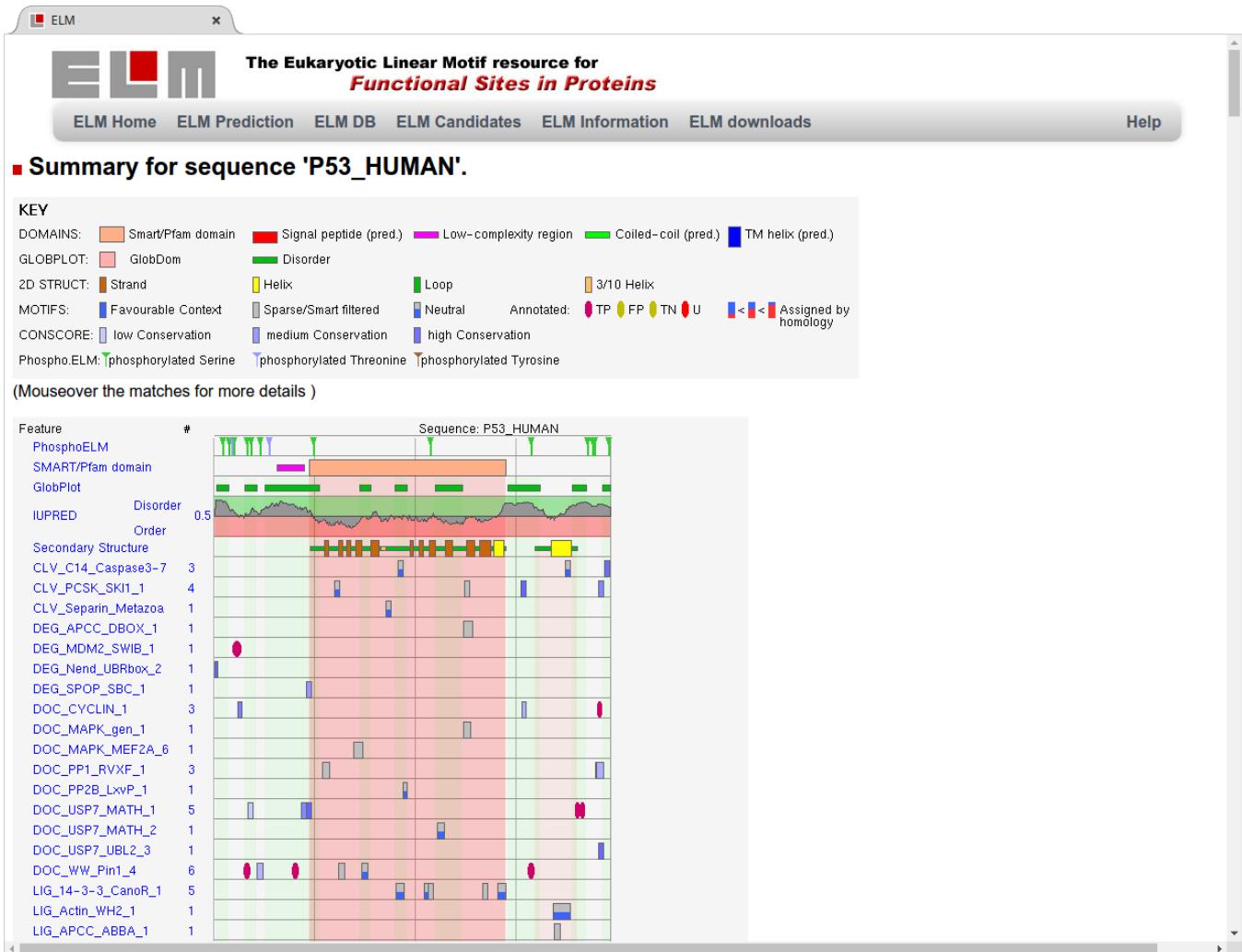
- ELM database update  
We have added new instances for: [LIG\\_APCC\\_ABBA\\_1](#), [LIG\\_APCC\\_ABBAvCdc20\\_2](#) as well as [DOC\\_MAPK\\_HePTP\\_8](#), [DOC\\_MAPK\\_MEF2A\\_6](#) and [DOC\\_MAPK\\_DCC\\_7](#)
- ELM Database Update  
We have updated several MOD\_CDk motifs and added new instances:  
MOD\_CDk\_1 is now: [MOD\\_CDk\\_SPxK\\_1](#), [MOD\\_CDk\\_SPK\\_2](#), [MOD\\_CDk\\_SPxxK\\_3](#) have been added.
- ELM database update  
Several new ELM classes and instances have been added:  
[LIG\\_BH\\_BH3\\_1](#), [DEG\\_COP1\\_1](#)
- ELM database update  
The class DOC\_PP2A\_KARD\_1 has been replaced by [DOC\\_PP2A\\_B56\\_1](#), and new instances have been added.
- ELM database update  
Several new ELM classes and instances have been added:  
[LIG\\_CSK\\_EPIYA\\_1](#), [LIG\\_Rb\\_LxCxE\\_1](#), [DOC\\_MAPK\\_JIP1\\_4](#), [DOC\\_MAPK\\_NFAT4\\_5](#)
- ELM database update  
Several new ELM classes and instances have been added:  
[DOC\\_MAPK\\_RevD\\_3](#), [LIG\\_ANK\\_PxPxL\\_1](#), [LIG\\_CSL\\_BTD\\_1](#), [LIG\\_G3BP\\_FGDF\\_1](#), [LIG\\_KLC1\\_TPR\\_1](#), [LIG\\_PALB2\\_WD40\\_1](#), [LIG\\_UFM1\\_UFIM\\_1](#)

Figure 22: **Figure TP53-BP1-1** The query input page for ELM for predicting motifs in a given protein sequence.

(note: this requires the Java browser plugin, which might not be available on some browsers). Alternatively you can also download the “alignment”, “conservation features” and “phosphosite features” files separately to view on a desktop (non-browser) installation of Jalview (Waterhouse et al. (2009)).

*The search for possible homologs is performed against the UniRef90 database, a dataset of protein sequences with less than 90 percent identity between any two of them (Suzek et al. (2007)). It is also possible that the BLAST results are not finished when the results page is shown: We suggest to refresh the page if you see the message “Either not enough data available to calculate a sequence alignment or the calculations haven’t finished yet”. In some cases it is also possible that no homologs will be detected. If you have refreshed the page after waiting for more than 3 minutes, this is most likely the case.*

12. Scroll down to the section titled “Filtering Summary” to view some statistics about how many motifs and instances were filtered out (figure TP53-BP1-2). The first two lines contain information on whether and which filters were applied in step XXX of this protocol. The next two lines (SMART &



**Figure 23: Figure TP53-BP1-2** The graphical results summary of the ELM Prediction pipeline for “P53\_HUMAN”. Note that not all motif detections are shown (the image is truncated at the bottom). The top five rows show a set of structural features. Annotated and predicted motifs are shown as differently colored ovals/boxes.

Structural score) show how many motifs and instances were removed by the SMART and Secondary structure filters. The “Retained by” section shows how many motif hits were not filtered out by the “Smart” or “Structural Score” filter. In this example a total of XXX instances (of XXX different motifs were identified), of which XXX instances (and XXX motifs) were filtered out as they occurred in a SMART domain.

*Note that the graphical summary above does not contain sequences filtered out by the “cell compartment” and “taxonomic context” filters (in step XXX). However those filtered out by the SMART and Structural scores are shown in the graphic above (as gray rectangles). If any “cell compartment” or “taxonomic context” filters are selected in step XXX, the number of motifs and instances are also shown in this table.*

13. On the results page, scroll down to the heading: “The ELMs in the following table are known

**Filtering summary**

User supplied cellular location(s): *nucleus, cytosol, endoplasmic reticulum, mitochondrion, Cytoplasm*  
User supplied taxon: *Homo sapiens*

(An ELM is listed as filtered when all its matching instances have been filtered out.)

		Elms	Instances
<b>FILTERED BY:</b>	<b>Species</b>	4	26
	<b>Cellular location</b> (counts only those ELMs not already excluded by species.)	5	11
	<b>Structural score</b> (below medium threshold score)	8	29
	<b>Smart</b> (in a domain and no structural filter info available)	0	0
<b>TOTAL FILTERED:</b>		17	66
<b>RETAINED BY:</b>	<b>Smart</b> (outside domain and no structural filter info available)	12	48
	<b>Structural score</b> (at or above medium threshold score)	32	58
<b>TOTAL RETAINED:</b>		44	106
<b>TOTAL</b>	<b>all found</b> (before filtering)	61	172

Query sequence:  
**>P53\_HUMAN**  
MEEPQSDPSVEPPLSQETFSDLWKLLEPNNVLSPLPSQAMDDMLSPDDIEQWFTEDPGP  
DEAPRMPEAAPPVAPAPAAPTAAAPAPSPWLSSVSPSQKTYQGSGYFRLGFLHSGTAK  
SVTCCTYSPALNKMFCQLAKTCPVOLWVDSTPPGTRVRAMAIVKOSQHMTEVVRRCPHHE  
RCSDSDGLAPPOHQHLRVEGNLRLRVEYLDDRNTRHSVVVPYEPPEVGSDCTIHZNYMCNS  
SCMGGMNRPIILTIITLEDSSSGNLGRNSFEVRVCACPGRDRRTTEENLRKKGEPHHELP  
PGSKRALPNTSSSOPKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAKGKEPG  
GSRAHSSHLSKKGQSTSRRHKKLMFKTEGPDSD

---

**Globular domains/ TM domains and signal peptide detected by the SMART server**

Domain	Start	End
Pfam:P53	95	289

---

**The ELMs in the following table are known instances annotated from the literature.**

Click on the link at positions to see experimental evidence.

Elm Name	Instances (Matched Sequence)	Positions	Logic	Elm Description	Cell Compartment	Pattern
MOD_PIKK_1	PPLSQET	12-18	true positive	(ST)Q motif which is phosphorylated by PIKK family members.	nucleus	...([ST])Q...
DOC_USP7_MATH_1	PGGSR AHSSH	359-363 364-368	true positive true positive	The USP7 MATH domain binding motif variant based on the MDM2 and p53 interactions.	nucleus	[PA][^P][^FYWIL]S[^P]
DEG_MDM2_SWIB_1	FSDLWKLL	19-26	true positive	An amphipathic $\alpha$ -helix found in p53 family members that binds in the hydrophobic cleft of MDM2's SWIB domain.	nucleus, cytosol	F[^P](3)W[^P](2,3)[VIL]

Figure 24: **Figure BACT-BP-3:** This section of the results contains additional details of alignment of homologous proteins, filtering results and globular domains.

instances annotated from the literature” (25). This table has details of SLiMs which have been manually annotated in the ELM database. The columns show each motif name, the sequence(s) that matched the motif as well as their starting and ending positions and the logic of the annotation followed by a short description of each motif, to which cell compartments its has been associated, and finally the regular expression of the motif.

*The “Logic” column indicates whether this motif is an example of a functional (True Positive, TP) or non-functional (False Positive, FP) motif. A TP instance is an instance annotated with experimental evidence showing this instance to be functional, whereas a FP is an instance with experimental evidence hinting at a function, but after careful inspection our annotators believe this instance to be non-functional. There are only rare cases of a true negative (TN) instance, which is an annotated instance where experiments have shown it to be non-functional.*

TODO: INSERT/CHANGE FIGURE/NAME

The ELMs in the following table are known instances annotated from the literature.

Click on the link at positions to see experimental evidence.

Elm Name	Instances (Matched Sequence)	Positions	Logic	Elm Description	Cell Compartment	Pattern
MOD_PIKK_1	PPLSQET	12-18	true positive	(ST)Q motif which is phosphorylated by PIKK family members.	nucleus	...([ST])Q...
DOC_USP7_MATH_1	PGGSR AHSSH	359-363 364-368	true positive true positive	The USP7 MATH domain binding motif variant based on the MDM2 and p53 interactions.	nucleus	[PA][^P][FYWIL]S[^P]
DEG_MDM2_SWIB_1	FSDLWKLL	19-26	true positive	An amphipatic $\alpha$ -helix found in p53 family members that binds in the hydrophobic cleft of MDM2's SWIB domain.	nucleus, cytosol	F[^P](3)W[^P](2,3)[VIL]
DOC_CYCLIN_1	KKLMF	381-385	true positive	Substrate recognition site that interacts with cyclin and thereby increases phosphorylation by cyclin/cdk complexes. Predicted proteins should have a CDK phosphorylation site. Also used by cyclin/cdk inhibitors.	cytosol, nucleus	[RK].L.(0,1)[FYLIVMP]
TRG_NLS_Bipartite_1	KRALPNNTSSSPQPKKPL	305-323	true positive	Bipartite variant of the classical basically charged NLS.	nucleus, Nuclear pore, NLS-dependent protein nuclear import complex	[KR][KR].(7,15)[^DE] ((K[RK])(RK))(([^DE] [KR]))((KR)[^DE]))[^DE]
MOD_CK1_1	SQETFSD	15-21	true positive	CK1 phosphorylation site	cytosol, nucleus	S..([ST])...
MOD_SUMO_for_1	FKTE	385-388	true positive	Motif recognised for modification by SUMO-1	nucleus, PML body	[VILMAFP](K).E
MOD_GSK3_1	NVLSPPLPS	30-37	true positive	GSK3 phosphorylation recognition site	cytosol, nucleus	...([ST])...[ST]
DOC_WW_Pin1_4	NVLSPPL AAPTPA TSSSPQ	30-35 78-83 312-317	true positive true positive true positive true positive	The Class IV WW domain interaction motif is recognised primarily by the Pin1 phosphorylation-dependent prolyl isomerase.	cytosol, nucleus	...([ST])P.
TRG_NES_CRM1_1	EMFRELNEALELKD	339-352	true positive	Some proteins re-exported from the nucleus contain a Leucine-rich nuclear export signal (NES) binding to the CRM1 exportin protein.	nucleus, cytosol	((DEQ).(0,1)[LIM].(2,3)[LVMF][^P](2,3)[LMVF].[LMIV].{0,3}[DE]) ((DE), (0,1)[LIM].(2,3)[LVMF][^P](2,3)[LMVF].[LMIV].{0,3}[DEQ])

Figure 25: Figure TP53-BP1-3

14. Scroll down to the section with the header “Globular domains/ TM domains and signal peptide detected by the SMART server” (Figure BACT-BP-3). This section contains information on which domains were detected by the SMART server, and their positions. Clicking on their names will bring you to the SMART entry for that domain on the SMART homepage.

TODO: INSERT/CHANGE FIGURE/NAME

TODO: INSERT/CHANGE FIGURE/NAME

15. Scroll further down to the section title “Results of ELM motif search after globular domain filtering, structural filtering and context filtering” to obtain an overview of all of the motifs and motif instances detected (26) Each row also contains information on the Motif name, the matching peptide sequence and its position. Additional information is shown about the ELM, cell compartment and its regular expression. If the motif was detected in a homologue, the column called “PHI-Blast Instance mapping” contains links to the Sequence alignment of the homologous protein, and a summary of the ELM instance mapper output. If a motif instance has been filtered out due to Structural

■ Results of ELM motif search after globular domain filtering, structural filtering and context filtering.

Matches falling inside globular protein domains are excluded from this list unless having an acceptable structural score (if the structural filter (BETA version) is applicable). If the structural filter (BETA version) is applicable it is possible to view these structures with Jmol

Elm Name	Instances (Matched Sequence)	Positions	View in Jmol	Elm Description	Cell Compartment	Pattern	PHI-Blast Instance Mapping	Structural Filter Info	
CLV_C14_Caspase3-7	SDSDG ELKDA EGPDS	183-187 [A] 349-353 [A] 388-392 [A]	183-187 349-353 -	Caspase-3 and Caspase-7 cleavage site.	cytosol, nucleus	[DSTE][^P] [^DEWHFYC]D[GSAN]	-	Output	3.094e-03
CLV_Separin_Metazoa	EVVRR	171-175 [A]	171-175	Separase cleavage site, best known in sister chromatid separation.	centrosome, nucleus, cytosol	E[IMPVL][MLVP]R.	-	Output	3.410e-04
DEG_MDM2_SWIB_1	FSDLWKLL	19-26	-	An amphipatic $\alpha$ -helix found in p53 family members that binds in the hydrophobic cleft of MDM2's SWIB domain.	nucleus, cytosol	F[^P](3)W(^P)(2,3)[VIL]	Output Summary	-	2.125e-05
DEG_SPOP_SBC_1	PLSSS	92-96 [A]	92-96	The S/T rich motif known as the SPOP-binding consensus (SBC) of the MATH-BTB protein, SPOP, is present in substrates that undergo SPOP/Cul3-dependent ubiquitination.	nuclear speck, nucleus, Cul3-RING ubiquitin ligase complex	[AVP].[ST][ST][ST]	-	Output	9.380e-04
DOC_CYCLIN_1	KLLP RALP KKLMF	24-27 [A] 306-309 [A] 381-385	- - -	Substrate recognition site that interacts with cyclin and thereby increases phosphorylation by cyclin/cdk complexes. Predicted proteins should have a CDK phosphorylation site. Also used by cyclin/cdk inhibitors.	cytosol, nucleus	[RK].L.(0,1)[FYLIVMP]	Output Summary	-	5.324e-03
DOC_PP1_RVXF_1	RHKKLMFK HKKLMFK	379-386 [A] 380-386 [A]	- -	Protein phosphatase 1 catalytic subunit (PP1c) interacting motif binds targeting proteins that dock to the substrate for dephosphorylation. The motif defined is [RK]{0,1}[VI][^P][FW].	nucleus, protein phosphatase type 1 complex, cytosol	.[RK]{0,1}[VIL][^P][FW].	-	-	8.301e-04
DOC_PP2B_LxvP_1	LAPP	188-191 [A]	188-191	Docking motif in calcineurin substrates that binds at the interface of the catalytic CNA and regulatory CNB subunits.	cytosol, calcineurin complex, nucleus	L.[LIVAPM]P	-	Output	2.296e-03
DOC_USP7_MATH_1	PLPSQ PAPSW PLSSS	34-38 [A] 87-91 [A] 92-96 [A]	- - 92-96	The USP7 MATH domain binding motif variant based on the MDM2 and p53 interactions.	nucleus	[PA][^P][^FYWIL]S[^P]	Output Summary	Output	1.239e-02

Figure 26: **Figure BACT-BP-7:** This table contains the list of motifs detected in the sequence (only the top part of the table is shown).

criteria (SMART or Structure), this column contains a link to a page with details on how individual criteria that make up this filter. The last column contains information on the Probability filter: the probability reflects the chance to observe this motif in any random amino acid sequence.

TODO: INSERT/CHANGE FIGURE/NAME

TODO: INSERT/CHANGE FIGURE/NAME

16. Scroll further down to the heading “List of excluded ELMs falling inside SMART/PFAM domains and/or scoring poorly with the structural filter (if applicable).” (Fig. ??) This table is (almost) identical to the one above, but shows motif instances which were rejected by the Structural filter or SMART filter.

## Protocol 4 (Alternate) Predicting ELMs in novel sequences

TODO: DESCRIBE MOST PROBABLE MOTIF INSTANCES (COMPARED TO FILTERED)

■ List of excluded ELMs falling inside SMART/PFAM domains and/or scoring poorly with the structural filter (if applicable).

Matches in this list are only likely to be of interest if they are in accessible surface-exposed loops. Motif matches buried in stably folded cores of globular domains are not plausible candidates.

If the structural filter (BETA version) is applicable it is possible to view these structures with Jmol. For more info consult the PDB structure entry used for structure filtering or the SMART or PFAM entries for useful links to solved 3D structures.

Elm Name	Positions	View in Jmol	Elm Description	Cell Compartment	Pattern	PHI-Blast Instance Mapping	Structural Filter Info	Probability
DEG_APCC_DBOX_1	248-256 [A]	248-256	An RxxL-based motif that binds to the Cdh1 and Cdc20 components of APC/C thereby targeting the protein for destruction in a cell cycle dependent manner	nucleus, cytosol	.R..L..[LIVM].	-	Output	
DOC_MAPK_gen_1	248-254 [A]	248-254	MAPK interacting molecules (e.g. MAPKKs, substrates, phosphatases) carry docking motif that help to regulate specific interaction in the MAPK cascade. The classic motif approximates (R/K)xxxx# where # is a hydrophobic residue.	nucleus, cytosol	[KR]{0,2}[KR], {0,2}[KR], {2,4} [ILVM].[ILVF]	-	Output	
DOC_MAPK_MEF2A_6	139-147 [A]	139-147	A kinase docking motif that mediates interaction towards the ERK1/2 and p38 subfamilies of MAP kinases.	cytosol, Transcription factor complex, nucleus	[RK]{2,4}[LIVMP].[LIV].[LIVMF]	-	Output	
DOC_PP1_RVXF_1	108-114 [A]	108-114	Protein phosphatase 1 catalytic subunit (PP1c) interacting motif binds targeting proteins that dock to the substrate for dephosphorylation. The motif defined is [RK]{0,1}[VI][^P][FW].	nucleus, protein phosphatase type 1 complex, cytosol	.[RK]{0,1}[VIL][^P][FW].	-	Output	
DOC_WW_Pin1_4	124-129 [A]	124-129	The Class IV WW domain interaction motif is recognised primarily by the Pin1 phosphorylation-dependent prolyl isomerase.	cytosol, nucleus	...((ST))P.	Output Summary	Output	
LIG_14-3-3_CanoR_1	213-217 [A] 267-271 [A]	213-217 267-271	Canonical Arg-containing phospho-motif mediating a strong interaction with 14-3-3 proteins.	cytosol, internal side of plasma membrane, nucleus	R[^DE]{0,2}[^DEPGI](ST)((FWYLMV).)[(^PRIKGN P)](^PRIKGN){2,4}[VILMFWYP])	-	Output	
LIG_APCC_ABBA_1	338-343 [A]	338-343	Amphipathic motif that is involved in APC/C inhibition by binding of CDH1/CDC20. In metazoan cyclin A, the motif also acts as a degron, enabling the cyclin's degradation in prometaphase.	spindle pole, nucleus, cytosol	[ILVMF].[ILMV]P[FHY].[DE]	-	Output	
LIG_FHA_1	128-144 [A]	128-	Phosphothreonine motif binding a subset of	nucleus	.(T).II.VI.	-	Output	

Figure 27: **Figure BACT-BP-8:** This table contains the list of motifs detected in the sequence (only the top part of the table is shown) which were excluded due to structural filters.

We will use protein “CV\_0974” (uniprot ID: Q7NZE8) as an example, a “probable tyrosine phosphatase” from *Chromobacterium violaceum*. This protein is predicted to be a tyrosine phosphatases because it has a “tyrosine phosphatase” (PTPc) domain.

## Protocol 4.1 Necessary Resources

### Protocol 4.1.1 Software & Hardware

A modern browser such as Firefox, Chrome, Safari. ELM is best viewed on a laptop or desktop computer, although tablets and smartphones will also work.

**ELM**

## ELM Prediction

The **ELM prediction** tool scans user-submitted protein sequences for matches to the regular expressions defined in ELM. Distinction is made between matches that correspond to experimentally validated motif instances already curated in the ELM database and matches that correspond to putative motifs based on the sequence. Since SLiMs are short and degenerate, overprediction is likely and many putative SLiMs will be false positives. However, predictive power is improved by using additional filters based on contextual information, including taxonomy, cellular compartment, evolutionary conservation and structural features.

**Protein sequence**

Enter Uniprot identifier or accession number: (auto-completion)  
e.g. **EPN1\_HUMAN, P04637, TAU\_HUMAN, [RANDOM]**

Or paste the sequence (Single letter code sequence only or FASTA format):  

```
>CV_0974
MSTIQTGIGLGGGRQLDLSRLDSLSGVNADKARIGIRKDGTLLVYTGRSYLLHPDQTRRADQFLKHLDLLIPGQKPREFRRLAQI
FDRPMALTQRNTQANETIARIPTQDVDTVRGGPKLWRADQAARPSGEPSRGERASLKQRNGAEHLKLQAPRAEAPRKH
DAIKTELASRLGSSDQPSGLLQLKAQVGSSAEGARFLNDVGQARFRDIPTAATQVRAPDGAPLPAKRQVQGGVNVIAISQY
PKAAQLESYFGMLAANRTPVLVVLASADAMAKQGRGKADLPDYFSQSGRYVEVESKSKGSTTLEGGLEVRAYHLNVRGAD
HKSVSIPLVHPNWADFEAQGATALKALAQHVDAVADKTTAFYRDNNSSALNDPDKLLPVIIHRAGVGRGTGQLIAABELLKPG
ASSLESIVADMGRSRNHLMVQTSGQLSTLVDLAQQQGRAILQPETAAEPIYANQQAEEPIYANDAPPPPRRRP
```

**Cell compartment (one or several):**

- not specified
- extracellular
- nucleus
- cytosol
- peroxisome
- glycosome
- glycosome
- Golgi apparatus
- endoplasmic reticulum
- lysosome
- endosome
- plasma membrane
- mitochondrion

**Taxonomic Context**

Type in species name (auto-completion):

**Motif Probability Cutoff:**

100

**Submit** **Reset Form**

**ELM DB**

The ELM relational database stores different types of data about experimentally validated SLiMs that are manually

**peptide from ELM class LIG\_PTB\_Apo\_2**

- ELM database update  
We have added new instances for: **LIG\_APCC\_ABBA\_1**, **LIG\_APCC\_ABBAvCdc20\_2** as well as **DOC\_MAPK\_HePTP\_8**, **DOC\_MAPK\_MEF2A\_6** and **DOC\_MAPK\_DCC\_7**
- ELM Database Update  
We have updated several MOD\_CDk motifs and added new instances:  
MOD\_CDk\_1 is now: **MOD\_CDk\_SPxK\_1**, **MOD\_CDk\_SPK\_2**, **MOD\_CDk\_SPxxK\_3** have been added.
- ELM database update  
Several new ELM classes and instances have been added:  
**LIG\_BH\_BH3\_1**, **DEG\_COP1\_1**
- ELM database update  
The class **DOC\_PP2A\_KARD\_1** has been replaced by **DOC\_PP2A\_B56\_1**, and new instances have been added.
- ELM database update  
Several new ELM classes and instances have been added:  
**LIG\_CSK\_EPIYA\_1**, **LIG\_Rb\_LxCxE\_1**, **DOC\_MAPK\_JIP1\_4**, **DOC\_MAPK\_NFAT4\_5**
- ELM database update  
Several new ELM classes and instances have been added:  
**DOC\_MAPK\_RevD\_3**, **LIG\_ANK\_PxPxL\_1**, **LIG\_CSL\_BTD\_1**, **LIG\_G3BP\_FGDF\_1**, **LIG\_KLC1\_TPR\_1**, **LIG\_PALB2\_WD40\_1**, **LIG\_UFM1\_UFM\_1**

Figure 28: **Figure BACT-BP-1:** The input query page for finding motifs in ELM. The sequence for *C. vilaceum* protein CV\_0974 was used as an example for this protocol.

## Protocol 4.2 Submitting a query to ELM

1. Click on the “ELM Predictions” button in the menu to access the search query page (Fig. 28). Here you can provide either a protein accession (from uniprot) or an amino acid sequence (simply the sequence, or a FASTA formatted entry) in which you want to detect SLiMs. Retrieve the FASTA formatted sequence from Uniprot (<http://www.uniprot.org/uniprot/Q7NZE8.fasta>), and enter it into the “sequence input text box”.  
**TODO: MENTION NOT TO USE “CHROMOBACTERIUM VIOLACEUM” IN THE ORGANISM BOX AND WHY**
2. The Results are summarized in the first figure on the results page (see Fig. 29) The Graphical summary shows all of the final and intermediate results generated by the ELM Prediction pipeline, and can be used infer whether or not a motif is present in a sequence, as well as how likely it is to be functional based on its structural context and evolutionary conservation.



Figure 29: **Figure BACT-BP-2:** The graphical results summary of the ELM Prediction pipeline for Probable Tyrosine phosphate (CV\_0974). Note that not all motif detections are shown (the image is truncated at the bottom). The top five rows show a handful of structural features. The motif occurrence are shown as blue boxes, the intensity of which indicates the conservation score. See steps XXX to YYY for more information.

3. Check the first row to see whether there are phosphorylation sites acid is a serine, threonine or tyrosine. In this case, no phosphorylation data could be found in the Phospho.ELM database ([Dinkel et al. \(2011\)](#)).
4. Check the second row showing SMART and Pfam domains. Hover the mouse over these domains to see their names and exact start and end positions.
5. The third row shows globular and disordered regions in the sequence as predicted by GlobPlot ([Linding et al. \(2003\)](#)). The 4th & 5th rows contain results from IUPred ([Dosztányi et al. \(2005\)](#)), another unstructured region prediction tool. Protein segments with an IUPred score above 0.5 are 95% likely to be disordered (REF???)

6. Place the cursor over the blue box for motif occurrence “DOC\_USP7\_MATH\_1” at position 129-133. This motif is in a disordered region, and has not been filtered out by the structural filter. However, its conservation score is extremely low: 0.000, indicating it is not conserved in homologous proteins. Place the cursor over motif “DOC\_MAPK\_DCC\_7” at positions “334-343”. Despite the high conservation score (1.000), this motif is inside the PTPc domain (and a Globular regions), and therefore has been filtered out.

TODO: CHECK CONSERVATION FILTER

### Protocol 4.3 Interpreting the prediction results: Additional Information

TODO: DESCRIBE HOW TO INTERPRETE THE PREDICTIONS USING THIS BACTERIAL EXAMPLE (OF WHICH NOT MUCH IS KNOWN). FOCUS ON HOW ONE SHOULD INTERPRETE THESE PREDICTIONS (LOOK AT DISORDER/GLOBULARITY, CONSERVATION)

## Protocol 5 (Alternate) Predicting ELMS in sequences using the API

Querying ELM for motifs in a given sequence (as discussed in basic protocol 1), gives you a nice overview of putative and possibly annotated motifs in your query protein with a graphical representation using colors to highlight different regions of the protein sequence (eg. disordered vs. globular). It is however difficult to analyse a large set of protein sequences in this manner. Therefore, <http://elm.eu.org> provides an interface which you can use to submit your sequence in a programmatic way. Of course, this way, you won’t receive the graphical output representation, but are limited to textual data representation.

Currently, there exists a single URL ‘[http://elm.eu.org/start\\_search/](http://elm.eu.org/start_search/)’ to accept such queries. You can choose to either submit a uniprot name or accession (ex. ‘[http://elm.eu.org/start\\_search/P53\\_HUMAN.tsv](http://elm.eu.org/start_search/P53_HUMAN.tsv)’) or submit your raw sequence (ex. ‘[http://elm.eu.org/start\\_search/MAPRGFSCLLLTSEIDLKVRRRA](http://elm.eu.org/start_search/MAPRGFSCLLLTSEIDLKVRRRA)’).

The logic here is, if the URL ends in ‘.tsv’ then the server assumes you are using a Uniprot id or accession; if it doesn’t, then it assumes you are using raw sequence. See below for details.

### Protocol 5.1 Necessary Resources

#### Protocol 5.1.1 Software

Ideally use `curl https://curl.haxx.se/` on the command line. This program can be launched from the terminal in any of the major operating systems: OSX, Windows and Linux. Of course `curl` is only one of many different ways to access web content programmatically, and we suggest anyone to use which ever program they feel is better suited for their tasks.

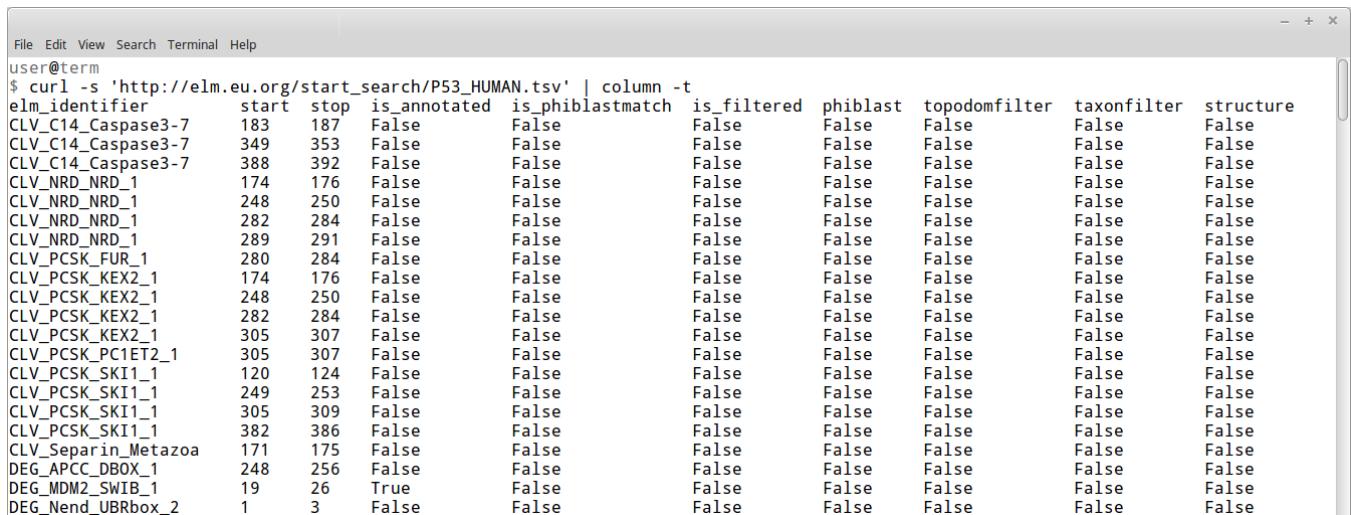
### Protocol 5.2 Submitting a query to ELM via the REST API

1. Use `curl` to query ELM for all motifs predicted to occur in Human P53 by typing the following into a terminal: ‘`curl ‘http://elm.eu.org/start\_search/P53\_HUMAN.tsv’`’. Each row represents a motif detection, and the first column “elm\_identifier” indicates which class was identified. The columns

“start” and “stop” show that first and last amino acid positions that matched form part of the motif. “is annotated” is True if this motif has been annotated in the database as an (experimentally validate) motif instance. “is phiblastmatch” is True if ??????. The column “is filtered” shows whether or not this motif was rejected by the ELM Prediction structure filter. “phibast” indicates whether ??????. The “topodomfilter” and “taxonfilter” shown whether ??????. The last column “structure” ??????

*In Figure ELM predictions pP53 we used a slightly more advanced command to get the output to look nice in the terminal. We specified the -s option to silence all curl output other than the downloaded file, and piped () the output directly to the column command (this command exists on most Linux and OSX machines).*

TODO: REDO FIGURE as it shows browser in transparent background



```
File Edit View Search Terminal Help
user@term
$ curl -s 'http://elm.eu.org/start_search/P53_HUMAN.tsv' | column -t
elm_identifier start stop is_annotated is_phiblastmatch is_filtered phiblast topodomfilter taxonfilter structure
CLV_C14_Caspase3-7 183 187 False False False False False False False
CLV_C14_Caspase3-7 349 353 False False False False False False False
CLV_C14_Caspase3-7 388 392 False False False False False False False
CLV_NRD_NRD_1 174 176 False False False False False False False
CLV_NRD_NRD_1 248 250 False False False False False False False
CLV_NRD_NRD_1 282 284 False False False False False False False
CLV_PCSK_FUR_1 289 291 False False False False False False False
CLV_PCSK_KEX2_1 280 284 False False False False False False False
CLV_PCSK_KEX2_1 174 176 False False False False False False False
CLV_PCSK_KEX2_1 248 250 False False False False False False False
CLV_PCSK_KEX2_1 282 284 False False False False False False False
CLV_PCSK_KEX2_1 305 307 False False False False False False False
CLV_PCSK_PC1ET2_1 305 307 False False False False False False False
CLV_PCSK_SKI1_1 120 124 False False False False False False False
CLV_PCSK_SKI1_1 249 253 False False False False False False False
CLV_PCSK_SKI1_1 305 309 False False False False False False False
CLV_PCSK_SKI1_1 382 386 False False False False False False False
CLV_Separin_Metazoa 171 175 False False False False False False False
DEG_APCC_DBOX_1 248 256 False False False False False False False
DEG_MDM2_SWIB_1 19 26 True False False False False False False
DEG_Nend_UBRbox_2 1 3 False False False False False False False
```

Figure 30: **Figure ELM Predictions P53:** The commandline output when curl is used to donload all motifs predicted in Human P53. Note that we used a more advanced command that curl alone to make the columns align nicely (see text for an explanation).

2. Use curl to query ELM via protein sequence by using the URL ‘[http://elm.eu.org/start\\_search/MAPRGFSCLL](http://elm.eu.org/start_search/MAPRGFSCLL)’ (Figure BACT-AP3-query). In this case the the query is an arbitrary short peptide sequence, but this can (of course) contain any sequence you are intersted in analysing. The output format is exactly the same as in the previous step.

*This way of querying ELM is unforntately not stable for long protein sequences. Different browsers and computers have different maximum lengths for URLs, and the excess text is often simply ignored. We reccomend not using this method for sequences longer than 2000 amino acids.*

TODo: REDO FIGURE as it shows browser in transparent background

TODo: add this information to the download page

TODo: maybe rename start\_search to query?

```

File Edit View Search Terminal Help
user@term
$ curl -s -o query.tsv 'http://elm.eu.org/start_search/P53_HUMAN.tsv'
user@term
$ head query.tsv | column -t
elm_identifier start stop is_annotated is_phiblastmatch is_filtered phiblast topodomfilter taxonfilter structure
CLV_C14_Caspase3-7 183 187 False False False False False False
CLV_C14_Caspase3-7 349 353 False False False False False False
CLV_C14_Caspase3-7 388 392 False False False False False False
CLV_NRD_NRD_1 174 176 False False False False False False
CLV_NRD_NRD_1 248 250 False False False False False False
CLV_NRD_NRD_1 282 284 False False False False False False
CLV_NRD_NRD_1 289 291 False False False False False False
CLV_PCSK_FUR_1 280 284 False False False False False False
CLV_PCSK_KEX2_1 174 176 False False False False False False
user@term
$ █

```

Figure 31: **Figure ELM Predictions on query sequence:** It is also possible to send amino acid sequences to the ELM Prediction pipeline. In this case we have used the curl option `-o` to download directly to the file `query.tsv`, and use a combination of the `head` and `column` commands to display the first 10 rows to the terminal.

## Protocol 6 (Alternate) Searching the ELM database using the REST API

Many researchers are interested in large-scale analyses rather than information about individual protein sequences. To this end, individual queries to the ELM webserver with a single protein id at a time, are not practical.

For this reason, as much information as possible is made available via a REST interface ([Fielding and Taylor \(2002\)](#)). This allows the user to interact with the ELM database and ELM webserver via scriptable URL requests. Each request can easily be tested in the browser before it is being automated in a script.

In this section we will explore the various ways in which data can be downloaded both in using the browser as well as via the commandline.

### Protocol 6.1 Necessary Resources

#### Protocol 6.1.1 Software

Ideally use `curl https://curl.haxx.se/` on the commandline. This program can be launched from the terminal in any of the major operating systems: OSX, Windows and Linux. Of course `curl` is only one of many different ways to access web content programmatically, and we suggest anyone to use whichever program they feel is better suited for their tasks.

The screenshot shows the ELM Downloads page. At the top, there's a navigation bar with links to ELM Home, ELM Prediction, ELM DB, ELM Candidates, ELM Information, ELM downloads, and Help. A search bar is also present. On the right, a sidebar lists categories: Classes, Instances, Interactions, Interaction Domains, Methods, PDBs, GOTerms, Renamed ELM classes, and Media / Files. The main content area has two tables. The first table, titled 'Classes' (last modified Dec. 7, 2016, 5:28 p.m.), shows examples like 'all' (html, tsv), 'by query term' (tsv), and 'by ELM id' (html). The second table, titled 'Instances' (last modified Dec. 8, 2016, 2:56 p.m.), shows various file formats (html, fasta, gff, tsv, pir, xml) for different types of instances.

Name	Example	URL
all	<a href="#">html</a>	/elms/elm_index.html
all	<a href="#">tsv</a>	/elms/elms_index.tsv
by query term	<a href="#">tsv</a>	/elms/elms_index.tsv?q=PCSK
by ELM id	<a href="#">html</a>	/ELME000012.html

Name	Example	URL
all	<a href="#">html</a>	/elms/instances.html?q=*
by Uniprot acc	<a href="#">fasta</a>	instances.fasta?q=P12931
by Uniprot name	<a href="#">gff</a>	instances.gff?q=SRC_HUMAN
by Uniprot acc	<a href="#">tsv</a>	instances.tsv?q=P12931
by query term	<a href="#">pir</a>	instances.pir?q=PCSK
by query term	<a href="#">tsv</a>	instances.tsv?q=src
by query term	<a href="#">mitab</a>	instances.mitab?q=src
by query term	<a href="#">xml</a>	instances.psimi?q=src
by query term using additional parameter "instance logic"	<a href="#">tsv</a>	instances.tsv?q=src&instance_logic=true+positive
by Instance id	<a href="#">html</a>	/ELMI000123.html
All docking motifs annotated in taxon "mouse"	<a href="#">tsv</a>	instances.tsv?q=DOC_&taxon=mus+musculus

Figure 32: **Figure ELM-Downloads:** The ELM downloads page, which holds information about the different types of data (such as “Classes”, “Instances”, etc; see menu to the right) that can be obtained from the server. The orange boxes are clickable links, the URL following them are used to highlight the URL scheme used by the server (bold font denotes specifics used in the examples such as query terms, or formats).

## Protocol 6.2 Downloading all ELM classes

1. Direct your browser to the URL ‘<http://elm.eu.org/downloads>’ or select ‘ELM Downloads’ from the main Menu (Figure ELM-Downloads) This webpage contains links and descriptions on how to download ELM data in text format. The datasets are split into several smaller collections (for example “Classes”, “Instances”, etc). Each table contains links (in orange) to download the data in various formats.

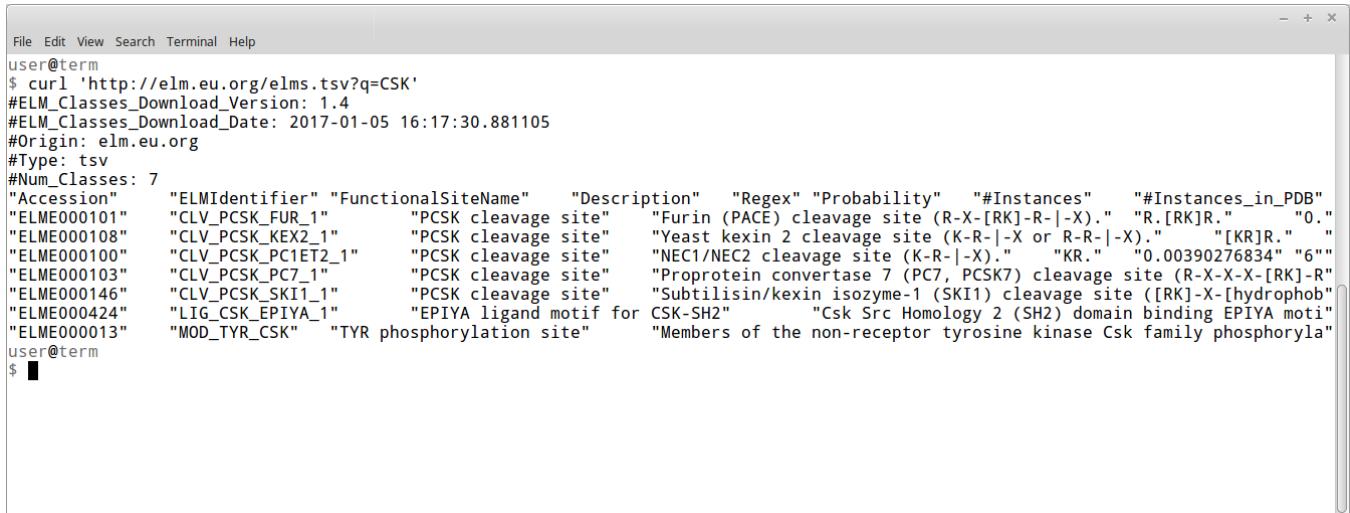
*Each table also shows the ‘last modified date’ indicating when the data was last updated. This is useful if you want to know when to update your local data with the most up to date ELM data.*

2. Click on the first orange ‘html’ link in the table “Classes” to navigate to the following URL:

‘[http://elm.eu.org/elms\(elm\\_index.html](http://elm.eu.org/elms(elm_index.html)’. This page shows all of the annotated ELM classes in the database. This page is the same one as shown in Figure *TP53-BP1-classes*

3. Navigated to the following URL: ‘<http://elm.eu.org/elms.html?q=CSK>’, specifying “q=CSK” to limit the list of ELMs to those matching the search query “CSK”. This page is again similar to the one shown in Figure *TP53-BP1-classes*, but with less classes.

*This search result is identical to the result you would obtain by doing a “manual” search on the ELM Classes page (<http://elm.eu.org/elms.html>). The column descriptions are also the same as described in Step XXX in Protocol YYY.*



```

File Edit View Search Terminal Help
user@term
$ curl 'http://elm.eu.org/elms.tsv?q=CSK'
#ELM_Classes_Download_Version: 1.4
#ELM_Classes_Download_Date: 2017-01-05 16:17:30.881105
#Origin: elm.eu.org
#Type: tsv
#Num_Classes: 7
"Accession" "ELMIdentifier" "FunctionalSiteName" "Description" "Regex" "Probability" "#Instances" "#Instances_in_PDB"
"ELME000101" "CLV_PCSK_FUR_1" "PCSK cleavage site" "Furin (PACE) cleavage site (R-X-[RK]-R|-X)." "R.[RK]R." "0."
"ELME000108" "CLV_PCSK_KEX2_1" "PCSK cleavage site" "Yeast kexin 2 cleavage site (K-R|-X or R-R|-X)." "[KR]R." "
"ELME000100" "CLV_PCSK_PC1ET2_1" "PCSK cleavage site" "NEC1/NEC2 cleavage site (K-R|-X)." "KR." "0.00390276834" "6"
"ELME000103" "CLV_PCSK_PC7_1" "PCSK cleavage site" "Protein convertase 7 (PC7, PCSK7) cleavage site (R-X-X-[RK])-R"
"ELME000143" "CLV_PCSK_SKI1_1" "PCSK cleavage site" "Subtilisin/kexin isozyme-1 (SKI1) cleavage site ([RK]-X-[hydrophob]"
"ELME000424" "LIG_CSK_EPIYA_1" "EPIYA ligand motif for CSK-SH2" "Csk Src Homology 2 (SH2) domain binding EPIYA motif"
"ELME000013" "MOD_TYR_CSK" "TYR phosphorylation site" "Members of the non-receptor tyrosine kinase Csk family phosphoryla"
user@term
$ █

```

Figure 33: **Figure ELM-Curl-Classes:** Screenshot of a terminal window using curl to download all ELM classes matching the term ‘CSK’.

4. Open the following URL: ‘<http://elm.eu.org/elms.tsv?q=CSK>’ to download a list of classes that match the search query “CSK” (as in the previous step) in the “tab separated values” format. By exchanging the ‘.html’ part of the url with ‘.tsv’, we ask the webserver to give us the data in TSV (tab-separated values) format.

*Depending on which browser you are using, the file may open directly in your browser, or you may be prompted to download the file or save it to a separate location. In the latter two cases you can open the downloaded file using a (plain) text file viewer, or possibly a spreadsheet viewer (such as Microsoft Excel or LibreOffice Calc).*

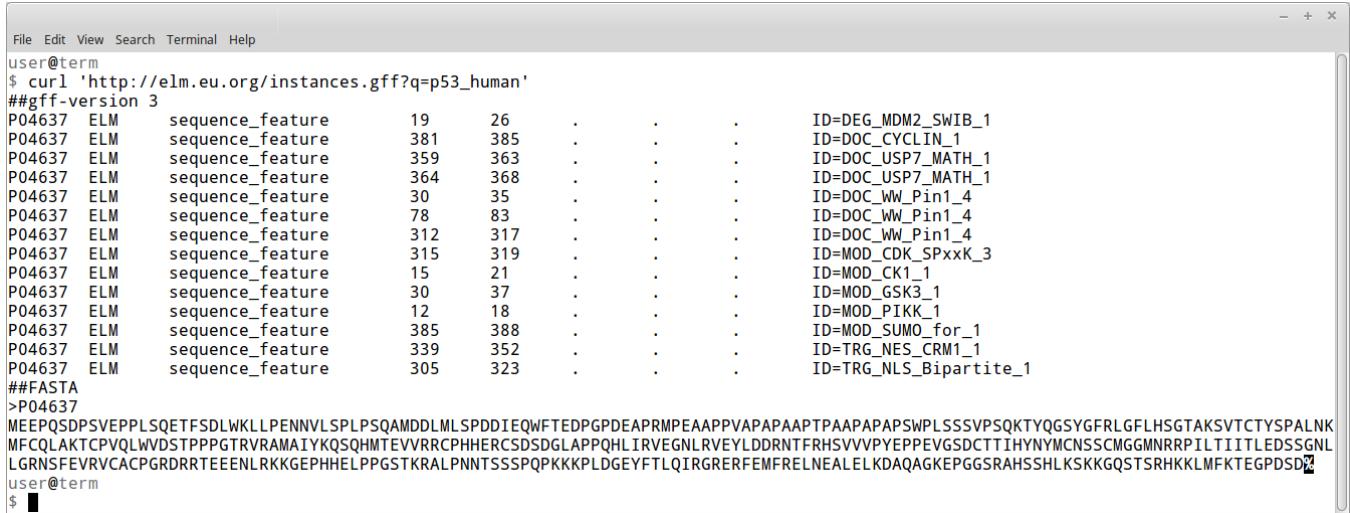
5. Type the following command into a command line terminal to download the same data from the previous step directly into the terminal: curl ‘[http://elm.eu.org/elms/elms\\_index.tsv?q=CSK](http://elm.eu.org/elms/elms_index.tsv?q=CSK)’’. The output should look similar to *Figure ELM-Curl-Classes*. The column names are still the same ones as shown in the *classes* table in Figure *BACT-AP2-Elm-classes-downloads*.

*Use the curl option -o to save the results directly to a file. For example: curl -o classes.tsv ‘[http://elm.eu.org/elms/elms\\_index.tsv?q=CSK](http://elm.eu.org/elms/elms_index.tsv?q=CSK)’ will save the data to a file called classes.tsv.*

6. To download a list of all motif instances detected in Human P53, type the following command into a terminal: curl ‘[http://elm.eu.org/instances.gff?q=p53\\_human](http://elm.eu.org/instances.gff?q=p53_human)’’. The output should look

similar to that shown in figure *Figure ELM-Curl-P53*. The output is in the “General Feature Format” (<http://www.ensembl.org/info/website/upload/gff.html#moreinfo>), with the FASTA formatted sequence appended to the end of the output.

*Many other file formats are available for downloading instances annotations, including the FASTA, GFF, PIR, or PSI-MI format (either XML or MiTab) Van Roey et al. (2013b).*



```

File Edit View Search Terminal Help
user@term
$ curl 'http://elm.eu.org/instances.gff?q=p53_human'
##gff-version 3
P04637 ELM sequence_feature 19 26 . . . ID=DEG_MDM2_SWIB_1
P04637 ELM sequence_feature 381 385 . . . ID=DOC_CYCLIN_1
P04637 ELM sequence_feature 359 363 . . . ID=DOC_USP7_MATH_1
P04637 ELM sequence_feature 364 368 . . . ID=DOC_USP7_MATH_1
P04637 ELM sequence_feature 30 35 . . . ID=DOC_WW_Pin1_4
P04637 ELM sequence_feature 78 83 . . . ID=DOC_WW_Pin1_4
P04637 ELM sequence_feature 312 317 . . . ID=DOC_WW_Pin1_4
P04637 ELM sequence_feature 315 319 . . . ID=MOD_CDK_SPXXK_3
P04637 ELM sequence_feature 15 21 . . . ID=MOD_CK1_1
P04637 ELM sequence_feature 30 37 . . . ID=MOD_GSK3_1
P04637 ELM sequence_feature 12 18 . . . ID=MOD_PIKK_1
P04637 ELM sequence_feature 385 388 . . . ID=MOD_SUMO_for_1
P04637 ELM sequence_feature 339 352 . . . ID=TRG_NES_CRM1_1
P04637 ELM sequence_feature 305 323 . . . ID=TRG_NLS_Bipartite_1
##FASTA
>P04637
MEEPQSDPSVEPPPLSQETFSDLWKLLEPENNVLSPPLPSQAMDDMLSPDDIEQWFTEDEPGPDEAPRMPEAAPPVAPAPAAPTAAAPAPAPSPLSSSVPSQKTYQGSYGRFLGFLHSGTAKSVTCTYSPLNK
MFCQLAKTCPVQLWDSTPPGTRVRAMAIYKQSQHMTEVRRCPHHERCSDSGDLAPPQHLIRVEGNLRVEYLDDRNTFRHSVVVPYEPPEVGSDCTTIHNYMCNSCMGGMNRRPILTIIITLEDSSGNL
LGRNSFEVRVCACPGDRRTEENLRKKGEPHHELPPGSTKRALPNNTSSSPQPKKPLDGEYFTLQIRGRERFEMFRELNEALELKDAQAGKEPGGSRAHSSHLSKKGQTSRHKKLMFKTEGPDS
user@term
$ 
```

Figure 34: **Figure ELM-Curl-Instances-P53:** Screenshot of a terminal window using `curl` to download all ELM instances annotated for sequence p53\_human.

7. To download a list of all instances matching the search query “CLV” in the yellow fever mosquito (*Aedes aegypti*), enter the following command into a terminal: ‘curl ‘[http://elm.eu.org/instances.tsv?q=CLV&taxon\\_id=1435](http://elm.eu.org/instances.tsv?q=CLV&taxon_id=1435)’’. In general any species name can be used, always replacing the “space” with a “+”. This should return a single instance, the only one matching CLV in *A. aegypti*.
  8. More data (interactions, domains, methods, etc.) can be downloaded from ELM in analogous fashion as shown in the preceding steps. Take a look at the ELM Downloads page (<http://elm.eu.org/downloads>, Figure *BACT-AP2-Elm-downloads*) for an overview of which datasets can be downloaded, and what the different possible filters and formats are for each dataset.
- % NOTE: TODO: Mention ELM software license agreement?

## Guidelines for Interpreting Results

*instructions: A brief discussion of the theory and applications of your*

*notes: Maybe mention how findings are relevant to the lab? For example: Manually annotated content should be reliable, although one should look at the ‘confidence’ in the instance annotation. Predictions are probably trustworthy, but you need to take into account the ‘confidence score’, and other features like whether its in a domain, etc...*

## **Commentary:**

*instructions: A brief discussion of the theory and applications of your*

### **Background Information**

In order to interpret the data contained in ELM and the results produced by the ELM prediction tool, it is important to have a basic understanding of SLiM's and how they are affected by their structural and biological context. This background information summarises the different functionalities of SLiMs, describes the degenerate nature of motif sequences, and emphasises the need for contextual data for confident SLiM prediction.

#### **ELM categorises SLiMs depending on their functionality**

SLiMs mediate different types of interactions, and based on this functionality, the ELM classes annotated in the ELM database are grouped into six main ELM types (Figure 1) ([Dinkel et al. \(2014\)](#)). They can function as ligand binding sites or as sites for post-translational modification (PTM). Some ligand SLiMs are recognised by components of the cellular transport machinery and function as localisation signals that target proteins to specific sub-cellular compartments (TRG type). Other ligand SLiMs are abundantly present in interfaces that mediate the assembly of large macromolecular complexes and in highly modular scaffold proteins that act as multivalent platforms for protein complex assembly (LIG type). Docking motifs are ligand SLiMs that recruit modification enzymes to their substrates by binding to a site on the enzyme that is distinct from the active site (DOC type). A subset of these, known as degrons, recruit ubiquitin ligases, which subsequently polyubiquitylate their substrates and hence target them for proteasomal degradation (DEG type). SLiMs that act as sites for PTM can be targeted by specific enzymes for the addition or removal of a small chemical group (e.g. phosphorylation), a sugar molecule (e.g. glycosylation), a protein (e.g. ubiquitylation), or another moiety (e.g. lipidation) (MOD type). Other PTM SLiMs mediate proteolytic cleavage by acting as target site for proteolytic enzymes (CLV type), or are recognised for structural modification by isomerases that catalyse cis-trans isomerisation of the peptide backbone (DOC type) [Van Roey et al. \(2014\)](#); [Lee et al. \(2015\)](#).

#### **ELM regular expressions reflect the degenerate nature of SLiMs**

As their name suggests, SLiMs are compact, being composed of a limited number of adjacent amino acids. Most of a motifâŽs binding specificity however is conferred by only a subset of these amino acids. Those few residues that directly interact with the binding partner are evolutionary conserved, although in many cases a subset of amino acids that share certain properties (such as similar charge, size or hydrophobicity) are allowed in these hotspot positions. In the motif positions that contribute little to the interaction, there are even less constraints, i.e. a broader range of amino acids is allowed in these positions [Davey et al. \(2012\)](#). This sequence flexibility is captured in the regular expressions that are defined for each motif class. A first consequence of this degeneracy is that SLiMs co-operatively engage in interactions of relatively low affinity. Hence these binding events are transient and reversible, and can be readily modulated, for instance by PTM. These characteristics make SLiM-based interactions ideal mediators of the dynamic processes involved in cell signalling [Van Roey et al. \(2012\)](#). Another consequence is that it might take only a few or even a single point mutation to generate or disrupt a functional motif in a protein. The

associated ability to evolve convergently might underlie the proliferation of SLiMs and the rewiring of interactomes [Davey et al. \(2015\)](#); [Kim et al. \(2012\)](#). Conversely, several SLiM-associated diseases have been characterised to date, for instance Liddle syndrome [Furuhashi et al. \(2005\)](#).

### **ELM integrates data to increase the confidence of SLiM prediction\***

Due to their degenerate nature, motif sequences contain only very little information, and many short sequences in a proteome will match motif patterns. However, most of these matches will not represent functional motifs, and hence, when scanning a proteome for putative motifs using only the motif sequence patterns will yield a large number of false positive instances, far exceeding the number of true motifs. Therefore, reliable motif detection cannot go without experimental validation of candidate motifs, using different types of experiments and techniques [Gibson et al. \(2015\)](#). This however does not mean that bioinformatics analysis cannot guide researchers towards a subset of candidate motifs that have a higher probability to be functional and help rule out those candidate motifs that are likely to be false positives. Taking into account additional information, besides a match to a sequence pattern defining a SLiM, can greatly narrow the selection of putative motifs for experimental validation. Additional data for in silico analysis include conservation of the motif sequence, the location of the motif within the proteinâŽs structure and its accessibility for its binding partner, validated interaction with the binding partner, and in-cell co-localisation with the binding partner. The availability and usefulness of these additional data for SLiM discovery depends on their extensive and correct biocuration. A vast and increasing amount of biological data is available in a wide variety of sources, including the literature and large-scale datasets. In order to facilitate integration of data, they need to be collected, annotated and formatted in central data and knowledge repositories. The ELM database provides such a repository for experimentally validated linear motif classes and instances. The ELM prediction tool in turn relies on annotated data, both from the ELM database and other resources, to accurately analyse unknown sequences for candidate motifs and assist researchers in selecting the most plausible ones for experimental validation and discard likely false positive hits, saving them valuable time and assets [Dinkel et al. \(2012\)](#).

## **Critical Parameters and Troubleshooting**

*instructions:* optionally 2 separate sections.

## **Internet Resources with Annotations**

<http://www.clustal.org/omega> Clustal Omega ([Sievers et al. \(2011\)](#)) is a tool for the alignment of multiple nucleic acid and protein sequences.

<http://www.jalview.org> Jalview ([Waterhouse et al. \(2009\)](#)) is a Java desktop application (and browser applet) that employs web services for sequence alignment and visualization.

<http://proviz.ucd.ie> ProViz ([Jehl et al. \(2016\)](#)) is an interactive protein exploration tool, which searches several databases for information about a given query protein. Data relevant to the protein like an alignment of homologues, linear motifs, post translational modifications, domains, secondary structure, sequence variations and others are graphically represented relative to their position in the protein.

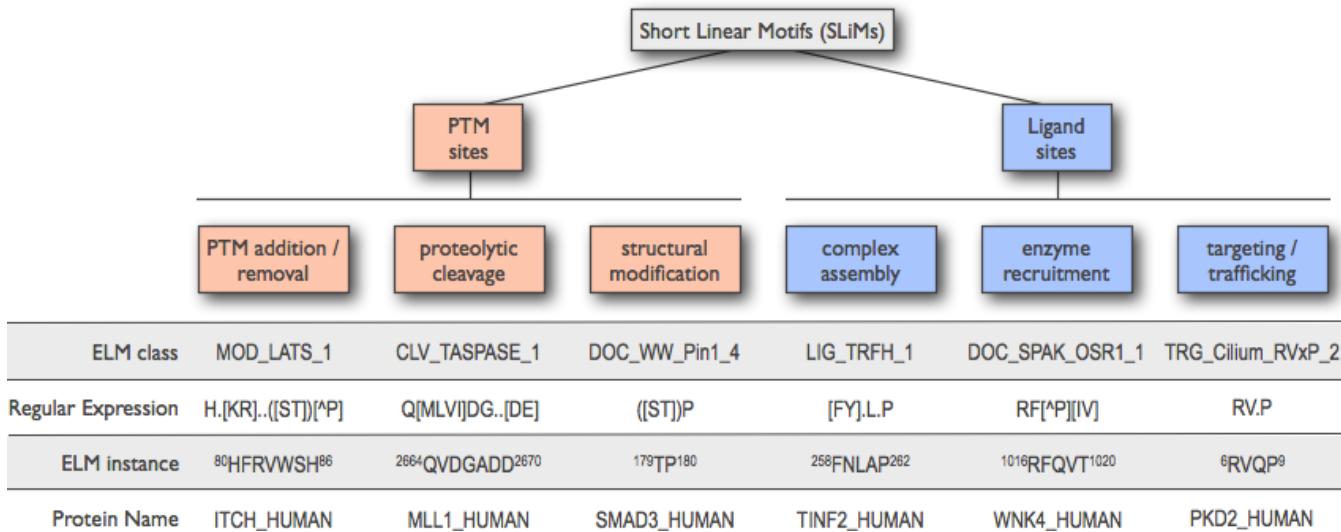


Figure 35: **Figure functional\_classification\_of\_SLiMs** For each ELM class, the functional category to which it belongs is indicated by a three-letter prefix. Each ELM class is defined by a regular expression. Peptide sequences in proteins that match the regular expression of a specific ELM class and that were experimentally validated to be functional motifs are captured as ELM instances of that class. Degrons are a specific subtype of enzyme-recruiting docking motifs (see text for a detailed description).

## References

- Berman, H. M., T. Battistuz, T. N. Bhat, W. F. Bluhm, P. E. Bourne, K. Burkhardt, Z. Feng, G. L. Gilliland, L. Iype, S. Jain, P. Fagan, J. Marvin, D. Padilla, V. Ravichandran, B. Schneider, N. Thanki, H. Weissig, J. D. Westbrook, and C. Zardecki. 2002. The protein data bank. *Acta crystallographica. Section D, Biological crystallography* 58:899–907.
- Chica, C., A. Labarga, C. M. Gould, R. López, and T. J. Gibson. 2008. A tree-based conservation scoring method for short linear motifs in multiple alignments of protein sequences. *BMC bioinformatics* 9:229.
- Consortium, T. G. O. 2017. Expansion of the gene ontology knowledgebase and resources. *Nucleic acids research* 45:D331–D338.
- Consortium, U. 2015. Uniprot: a hub for protein information. *Nucleic acids research* 43:D204–12.
- Coordinators, N. R. 2017. Database resources of the national center for biotechnology information. *Nucleic acids research* 45:D12–D17.
- Davey, N. E., M. S. Cyert, and A. M. Moses. 2015. Short linear motifs àÁ ex nihilo evolution of protein regulation. *Cell Communication and Signaling* 13:43.
- Davey, N. E., K. Van Roey, R. J. Weatheritt, G. Toedt, B. Uyar, B. Altenberg, A. Budd, F. Diella, H. Dinkel, and T. J. Gibson. 2012. Attributes of short linear motifs. *Molecular bioSystems* 8:268–81.
- Diella, F. 2008. Understanding eukaryotic linear motifs and their role in cell signaling and regulation. *Frontiers in Bioscience Volume:6580*.

- Dinkel, H., C. Chica, A. Via, C. M. Gould, L. J. Jensen, T. J. Gibson, and F. Diella. 2011. Phospho.elm: a database of phosphorylation sites—update 2011. *Nucleic acids research* 39:D261–7.
- Dinkel, H., S. Michael, R. J. Weatheritt, N. E. Davey, K. Van Roey, B. Altenberg, G. Toedt, B. Uyar, M. Seiler, A. Budd, L. Jödicke, M. A. Dammert, C. Schroeter, M. Hammer, T. Schmidt, P. Jehl, C. McGuigan, M. Dymecka, C. Chica, K. Luck, A. Via, A. Chatr-Aryamontri, N. Haslam, G. Grebnev, R. J. Edwards, M. O. Steinmetz, H. Meiselbach, F. Diella, and T. J. Gibson. 2012. Elm—the database of eukaryotic linear motifs. *Nucleic acids research* 40:D242–51.
- Dinkel, H., K. Van Roey, S. Michael, N. E. Davey, R. J. Weatheritt, D. Born, T. Speck, D. Krüger, G. Grebnev, M. Kubań, M. Strumillo, B. Uyar, A. Budd, B. Altenberg, M. Seiler, L. B. Chemes, J. Glavina, I. E. Sánchez, F. Diella, and T. J. Gibson. 2014. The eukaryotic linear motif resource ELM: 10 years and counting. *Nucleic Acids Research* 42:D259–D266.
- Dosztányi, Z., V. Csizmok, P. Tompa, and I. Simon. 2005. Iupred: web server for the prediction of intrinsically unstructured regions of proteins based on estimated energy content. *Bioinformatics (Oxford, England)* 21:3433–4.
- Fielding, R. T. and R. N. Taylor. 2002. Principled design of the modern web architecture. *ACM Transactions on Internet Technology* 2:115–150.
- Finn, R. D., T. K. Attwood, P. C. Babbitt, A. Bateman, P. Bork, A. J. Bridge, H.-Y. Chang, Z. Dosztányi, S. El-Gebali, M. Fraser, J. Gough, D. Haft, G. L. Holliday, H. Huang, X. Huang, I. Letunic, R. Lopez, S. Lu, A. Marchler-Bauer, H. Mi, J. Mistry, D. A. Natale, M. Necci, G. Nuka, C. A. Orengo, Y. Park, S. Pesceat, D. Piovesan, S. C. Potter, N. D. Rawlings, N. Redaschi, L. Richardson, C. Rivoire, A. Sangrador-Vegas, C. Sigrist, I. Sillitoe, B. Smithers, S. Squizzato, G. Sutton, N. Thanki, P. D. Thomas, S. C. E. Tosatto, C. H. Wu, I. Xenarios, L.-S. Yeh, S.-Y. Young, and A. L. Mitchell. 2017. Interpro in 2017—beyond protein family and domain annotations. *Nucleic acids research* 45:D190–D199.
- Finn, R. D., P. Coggill, R. Y. Eberhardt, S. R. Eddy, J. Mistry, A. L. Mitchell, S. C. Potter, M. Punta, M. Qureshi, A. Sangrador-Vegas, G. A. Salazar, J. Tate, and A. Bateman. 2016. The pfam protein families database: towards a more sustainable future. *Nucleic acids research* 44:D279–85.
- Furuhashi, M., K. Kitamura, M. Adachi, T. Miyoshi, N. Wakida, N. Ura, Y. Shikano, Y. Shinshi, K.-i. Sakamoto, M. Hayashi, N. Satoh, T. Nishitani, K. Tomita, and K. Shimamoto. 2005. Liddle's Syndrome Caused by a Novel Mutation in the Proline-Rich PY Motif of the Epithelial Sodium Channel  $\beta$ -Subunit. *The Journal of Clinical Endocrinology & Metabolism* 90:340–344.
- Gibson, T. J., H. Dinkel, K. Van Roey, and F. Diella. 2015. Experimental detection of short regulatory motifs in eukaryotic proteins: tips for good practice as well as for bad. *Cell Communication and Signaling* 13:42.
- Jehl, P., J. Manguy, D. C. Shields, D. G. Higgins, and N. E. Davey. 2016. Proviz—a web-based visualization tool to investigate the functional and evolutionary features of protein sequences. *Nucleic acids research* 44:W11–5.
- Kanehisa, M., Y. Sato, M. Kawashima, M. Furumichi, and M. Tanabe. 2016. Kegg as a reference resource for gene and protein annotation. *Nucleic acids research* 44:D457–62.

- Kerrien, S., S. Orchard, L. Montecchi-Palazzi, B. Aranda, A. F. Quinn, N. Vinod, G. D. Bader, I. Xenarios, J. Wojcik, D. Sherman, M. Tyers, J. J. Salama, S. Moore, A. Ceol, A. Chatr-Aryamontri, M. Oesterheld, V. Stümpflen, L. Salwinski, J. Nerothin, E. Cerami, M. E. Cusick, M. Vidal, M. Gilson, J. Armstrong, P. Woolland, C. Hogue, D. Eisenberg, G. Cesareni, R. Apweiler, and H. Hermjakob. 2007. Broadening the horizon—level 2.5 of the hupo-psi format for molecular interactions. *BMC biology* 5:44.
- Kim, J., I. Kim, J.-S. Yang, Y.-E. Shin, J. Hwang, S. Park, Y. S. Choi, and S. Kim. 2012. Rewiring of PDZ Domain-Ligand Interaction Network Contributed to Eukaryotic Evolution. *PLoS Genetics* 8:e1002510.
- Lee, R. V. D., M. Buljan, B. Lang, R. J. Weatheritt, G. W. Daughdrill, A. K. Dunker, M. Fuxreiter, J. Gough, J. Gsponer, D. T. Jones, P. M. Kim, R. W. Kriwacki, C. J. Old, R. V. Pappu, P. Tompa, V. N. Uversky, P. E. Wright, and M. M. Babu. 2015. Classification of Intrinsically Disordered Regions and Proteins. *Prog Biophys Mol Biol* .
- Letunic, I., T. Doerks, and P. Bork. 2015. Smart: recent updates, new developments and status in 2015. *Nucleic acids research* 43:D257–60.
- Linding, R., R. B. Russell, V. Neduvia, and T. J. Gibson. 2003. Globplot: Exploring protein sequences for globularity and disorder. *Nucleic acids research* 31:3701–8.
- McKusick, V. A. 2007. Mendelian inheritance in man and its online version, omim. *American journal of human genetics* 80:588–604.
- Schultz, J., F. Milpetz, P. Bork, and C. P. Ponting. 1998. Smart, a simple modular architecture research tool: identification of signaling domains. *Proceedings of the National Academy of Sciences of the United States of America* 95:5857–64.
- Sievers, F., A. Wilm, D. Dineen, T. J. Gibson, K. Karplus, W. Li, R. Lopez, H. McWilliam, M. Remmert, J. Söding, J. D. Thompson, and D. G. Higgins. 2011. Fast, scalable generation of high-quality protein multiple sequence alignments using clustal omega. *Molecular systems biology* 7:539.
- Suzek, B. E., H. Huang, P. McGarvey, R. Mazumder, and C. H. Wu. 2007. Uniref: comprehensive and non-redundant uniprot reference clusters. *Bioinformatics (Oxford, England)* 23:1282–8.
- Tompa, P., N. E. Davey, T. J. Gibson, and M. M. Babu. 2014. A million peptide motifs for the molecular biologist. *Molecular cell* 55:161–9.
- Van Roey, K., H. Dinkel, R. J. Weatheritt, T. J. Gibson, and N. E. Davey. 2013a. The switches.elm resource: a compendium of conditional regulatory interaction interfaces. *Science signaling* 6:rs7.
- Van Roey, K., T. J. Gibson, and N. E. Davey. 2012. Motif switches: decision-making in cell regulation. *Current opinion in structural biology* 22:378–85.
- Van Roey, K., S. Orchard, S. Kerrien, M. Dumousseau, S. Ricard-Blum, H. Hermjakob, and T. J. Gibson. 2013b. Capturing cooperative interactions with the psi-mi format. *Database : the journal of biological databases and curation* 2013:bat066.
- Van Roey, K., B. Uyar, R. J. Weatheritt, H. Dinkel, M. Seiler, A. Budd, T. J. Gibson, and N. E. Davey. 2014. Short linear motifs: ubiquitous and functionally diverse protein interaction modules directing cell regulation. *Chemical reviews* 114:6733–78.

- Via, A., C. M. Gould, C. Gemünd, T. J. Gibson, and M. Helmer-Citterich. 2009. A structure filter for the eukaryotic linear motif resource. *BMC bioinformatics* 10:351.
- Waterhouse, A. M., J. B. Procter, D. M. A. Martin, M. Clamp, and G. J. Barton. 2009. Jalview version 2—a multiple sequence alignment editor and analysis workbench. *Bioinformatics (Oxford, England)* 25:1189–91.
- Wright, P. E. and H. J. Dyson. 1999. Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. *Journal of molecular biology* 293:321–31.