OrganelX Web Server for Sub-Peroxisomal and Sub-Mitochondrial protein localisation

Marco Anteghini[1,2,*,†], Asmaa Haja[3,*,†], Vitor AP Martins dos Santos[1,2], Lambert Schomaker[3], Edoardo Saccenti[2]

1 LifeGlimmer GmbH, Berlin, Germany; **2** Laboratory of Systems and Synthetic Biology, Wageningen University & Research, Wageningen WE, The Netherlands; **3** Bernoulli Institute, University of Groningen, Groningen, The Netherlands.

[*]To whom correspondence should be addressed.
[†] The authors wish it to be known that, in their opinion, the first two authors should be regarded as Joint First Authors

Contact: marco.anteghini@wur.nl; P.O. Box 8033, 6700 EJ Wageningen, The Netherlands

Abstract

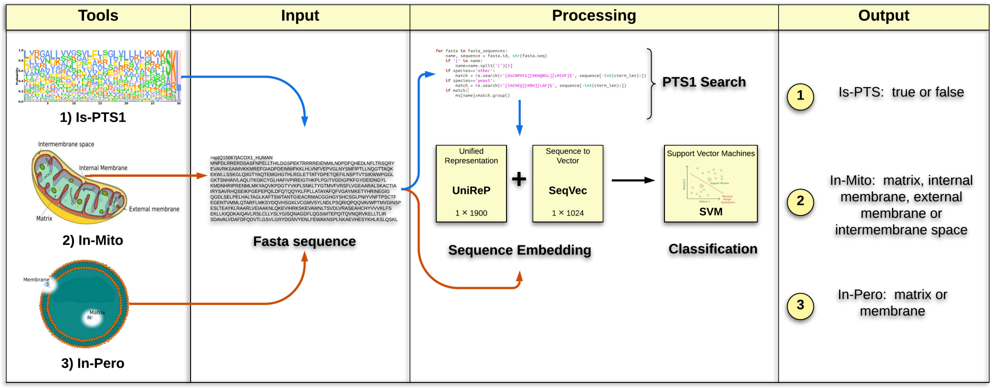
Computational approaches for sub-organelle protein localisation and identification are often neglected while general methods, not suitable for specific use cases, are promoted instead. In particular, organelle-specific research lacks user-friendly and easily accessible computational tools that allow researchers to perform computational analysis before starting time-consuming and expensive wet-lab experiments. We present the Organelx e-Science Web Server which hosts three sequence localisation predictive algorithms: In-Pero and In-Mito for classifying sub-peroxisomal and sub-mitochondrial protein localisations given their FASTA sequences, as well as the Is-PTS1 algorithm for detecting and validating potential peroxisomal proteins carrying a PTS1 signal. These tools can be used for a fast and accurate screening while looking for new peroxisomal and mitochondrial proteins. To our knowledge, this is the only service that provides these functionalities and can fasten the daily research of the peroxisomal science community.

Keywords: subcellular localisation, sub-peroxisomal localisation, sub-mithocondrial localisation, Web Server, protein-targeting-signal prediction

1 Introduction

Signatures in the amino acid composition of proteins have been associated with protein domains, families functional sites and their subcellular localisation [1–4]. This information can be combined with machine learning (ML) approaches to develop prediction tools, that nowadays are easily findable and accessible [5–8].

Moreover, ML and deep-learning (DL) approaches are often used to encode protein sequences, thus showing promising results in several tasks, including subcellular classification [9–15]. Unified Representation (UniRep) [9] and the Sequence-to-Vector (SeqVec) [10] are two of most promising and already used DL-embeddings embeddings.



```
>sp|Q15067|ACOX1_HUMAN
MNPDLRRERDSASFNPELLTHILDGSPEKTRRRREIENMILNDPDFQHEDLNFLTRSQRY
EVAVRKSAIMVKKMREFGIADPDEIMWFKKLHLVNFVEPVGLNYSMFIPTLLNQGTTAQK
EKWLLSSKGLQIIGTYAQTEMGHGTHLRGLETTATYDPETQEFILNSPTVTSIKWWPGGL
GKTSNHAIVLAQLITKGKCYGLHAFIVPIREIGTHKPLPGITVGDIGPKFGYDEIDNGYL
KMDNHRIPRENMLMKYAQVKPDGTYVKPLSNKLTYGTMVFVRSFLVGEAARALSKACTIA
IRYSAVRHQSEIKPGEPEPQILDFQTQQYKLFPLLATAYAFQFVGAYMKETYHRINEGIG
QGDLSELPELHALTAGLKAFTSWTANTGIEACRMACGGHGYSHCSGLPNIYVNFTPSCTF
EGENTVMMLQTARFLMKSYDQVHSGKLVCGMVSYLNDLPSQRIQPQQVAVWPTMVDINSP
ESLTEAYKLRAARLVEIAAKNLQKEVIHRKSKEVAWNLTSVDLVRASEAHCHYVVVKLFS
EKLLKIQDKAIQAVLRSLCLLYSLYGISQNAGDFLQGSIMTEPQITQVNQRVKELLTLIR
SDAVALVDAFDFQDVTLGSVLGRYDGNVYENLFEWAKNSPLNKAEVHESYKHLKSLQSKL
```

```
for fasta in fasta_sequences:
    name, sequence = fasta.id, str(fasta.seq)
    if '|' in name:
        name=name.split('|')[1]
    if species=='other':
        match = re.search(r'[ASCNPHTG][RKHQNSL][LMIVF]$', sequence[-int(cterm_len):])
    if species=='yeast':
        match = re.search(r'[SACHEQ][KRH][LAF]$', sequence[-int(cterm_len):])
    if match:
        ms[name]=match.group()
```

Figure 1. Web Server tools overview. The 4 columns show the typical workflow which consists of: (1) tool selection (Is-PTS1, In-Pero, In-Mito); (2) input a FASTA file containing one or more sequences; (3) processing via sequence embedding representation and SVM classification; (4) output generation.

UniRep [9] provides amino-acid embedding containing meaningful physicochemically and phylogenetic clusters and proved to be efficient for distinguishing proteins from various SCOP (structural classifications of proteins) classes. SeqVec showed similar results and optimal performance for predicting subcellular localisation [10]. However, their potential has been explored for highly specific tasks, such as sub-organelle localisation, just recently [16]. In particular, their usage can be adapted for sub-peroxisomal and sub-mitochondrial protein localisation.

Peroxisomes and mitochondria are ubiquitous organelles surrounded by a single biomembrane (peroxisomes) or a double biomembrane (mitochondria) that are relevant to many metabolic and non-metabolic pathways [17, 18]. However, the full extent of the functions of peroxisomes, mitochondria and their shared pathways is still largely unknown [19] and the discovery of new peroxisomal and mitochondrial proteins can facilitate further knowledge acquisition.

The OrganelX Web Server, available at https://organelx.hpc.rug.nl/fasta/, hosts two algorithms designed to classify sub-peroxisomal (In-Pero) and sub-mitochondrial (In-Mito) proteins localisations given their FASTA sequences. In addition, it is possible to perform a specific protein targeting signal (PTS) research in the sequence (Is-PTS1). Our tool consents to detect and validate on the PTS1 associated with peroxisomal proteins. The PTS1 is present in the C-terminal part of the protein sequence. It is defined as the final dodecamer with a focus on the terminal tripeptide [20]. An overview of the functionalities available in the Web Server is shown in Figure 1.

To our knowledge, there is no service that automatically performs In-Pero and In-Mito classification as well as Is-PTS1 detection. These predictions are noticeably useful for peroxisomal-related research [21].

2 OrganelX functionalities and examples

Input The input is a FASTA text file containing one or more protein sequences. Each sequence begins with a single-line description, followed by sequence data. The single-line description consists of **>sp|ID|Desc** symbols, where **>sp** is a fixed prefix, **ID** is the sequence name, and **Desc** is a descriptive text, followed by tokens of the FASTA sequence on the next lines.

Output The output file is a plain text file containing the classification results. The results of each class are represented by the predicted class name followed by ':' and the IDs of the corresponding classified entries.

Processing Methods The three classification algorithms start representing the sequence as a concatenation of two deep learning-based sequence embeddings, namely UniRep and Seqvec [9, 10]. The classification is performed by Support Vector Machines (SVM), where the In-Pero and In-Mito algorithms classify among sub-organelle classes. There are two classes (matrix and membrane) for peroxisomal proteins and four classes (matrix, inner-membrane, intermembrane, outer-membrane) for mitochondrial proteins. The third algorithm detects the PTS1 signal by matching a regular expression. Adopting a similar workflow that starts with the embedding representation and performs an SVM based classification, the Is-PTS1 validation algorithm classifies true and false PTS1.

Validation The algorithm was validated on two validation sets. Data were downloaded from Uniprot [22] on the 25/02/2021. For validating In-Pero, we queried for reviewed proteins with a clear sub-peroxisomal annotation in membrane (”SL-0203” and ”GO:0005778”) or matrix (”SL-0202” and ”GO:0005782”). We then clustered the query results for 40% of sequence identity with CD-hit [23] and removed the sequences overlapping with our training set, obtaining 85 membrane proteins and 59 matrix proteins. Our model obtained an accuracy of 0.88. We also tested the Is-PTS1 predictor retrieving in a similar way 15 true and 15 false, where true refers to a peroxisomal protein carrying the PTS1 signal and false refers to a peroxisomal protein without PTS1 signal. The performance in terms of accuracy is 0.83. The in-depth analysis of the In-Mito predictor is available in the original paper [16]. Datasets are available at https://github.com/MarcoAnteghini/.

Examples In order to submit a job, the user must specify an arbitrary username, his email address (optional) and upload a FASTA file. The computation time changes depending on the file size and the traffic on the website. The user can either wait for the results via email, or simply refreshing the result web page. An example is visible at https://organelx.hpc.rug.nl/fasta/test_example, where also a FASTA file can be downloaded.

Acknowledgements

We thank the Center for Information Technology of the University of Groningen for providing access to the Peregrine high performance computing cluster. The e-Science server was realized with the support and nurturing of Ger Strikwerda.

Funding

This project has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 812968.

Conflict of interest

The authors declare no conflict of interest.

Author Contribution

- Category 1:
  Conception and design of study: MA, AH, ES; acquisition of data MA, AH; analysis and/or interpretation of data: MA, AH, ES, VMS, LS.

- Category 2:
  Drafting the manuscript: MA, AH; revising the manuscript critically for important intellectual content: LS, ES, VMS.

- Category 3:
  Approval of the version of the manuscript to be published: MA, AH, VMS, LS, ES.

References

1. Andrade MA, O'Donoghue SI and Rost B. Adaptation of protein surfaces to subcellular location 1 1Edited by F. E. Cohen. Journal of Molecular Biology 1998; 276:517–525.

2. Hunter S, Apweiler R, Attwood TK et al. InterPro: the integrative protein signature database. Nucleic Acids Research 2009;37:D211–D215.

3. Scott MS, Thomas DY and Hallett MT. Predicting Subcellular Localization via Protein Motif Co-Occurrence. Genome Research 2004;14:1957–1966.

4. Almagro Armenteros JJ, Salvatore M, Emanuelsson O et al. Detecting sequence signals in targeting peptides using deep learning. Life Science Alliance 2019;2.

5. Horton P, Park KJ, Obayashi T et al. WoLF PSORT: protein localization predictor. Nucleic Acids Research 2007;35:W585–W587.

6. Pierleoni A, Martelli PL and Fariselli P. BaCelLo: a Balanced subCellular Localization predictor. Bioinformatics (Oxford, England) 2006;22:e408–16.

7. Savojardo C, Martelli PL, Fariselli P and Casadio R. TPpred3 detects and discriminates mitochondrial and chloroplastic targeting peptides in eukaryotic proteins. Bioinformatics 2015;31:3269–3275.

8. Jiang Y, Wang D, Yao Y et al. MULocDeep: A deep-learning framework for protein subcellular and suborganellar localization prediction with residue-level interpretation, 2020.

9. Alley E, Khimulya G, Biswas S et al. Unified rational protein engineering with sequence-based deep representation learning. Nature Methods 2019;16.

10. Heinzinger M, Elnaggar A, Wang Y et al. Modeling aspects of the language of life through transfer-learning protein sequences. BMC Bioinformatics 2019;20.

11. Elnaggar A, Heinzinger M, Dallago C et al. ProtTrans: Towards Cracking the Language of Life's Code Through Self-Supervised Deep Learning and High Performance Computing. bioRxiv 2020;.

12. Rives A, Meier J, Sercu T et al. Biological structure and function emerge from scaling unsupervised learning to 250 million protein sequences. Proceedings of the National Academy of Sciences 2021;118:e2016239118.

13. Savojardo C, Bruciaferri N, Tartari G et al. DeepMito: accurate prediction of protein sub-mitochondrial localization using convolutional neural networks. Bioinformatics 2019;36:56–64.

14. Almagro Armenteros JJ, Sønderby CK, Sønderby SK et al. DeepLoc: prediction of protein subcellular localization using deep learning. Bioinformatics 2017; 33:3387–3395.

15. Ho Thanh Lam L, Le NH, Van Tuan L et al. Machine Learning Model for Identifying Antioxidant Proteins Using Features Calculated from Primary Sequences. Biology 2020;9.

16. Anteghini M, dos Santos VM and Saccenti E. In-Pero: Exploiting Deep Learning Embeddings of Protein Sequences to Predict the Localisation of Peroxisomal Proteins. International Journal of Molecular Sciences 2021;22:6409.

17. Wanders RJA, Waterham HR and Ferdinandusse S. Metabolic Interplay between Peroxisomes and Other Subcellular Organelles Including Mitochondria and the Endoplasmic Reticulum. Frontiers in Cell and Developmental Biology 2016;3:83.

18. Islinger M, Voelkl A, Fahimi H and Schrader M. The peroxisome: an update on mysteries 2.0. Histochemistry and Cell Biology 2018;150:1–29.

19. Islinger M, Grille S, Fahimi HD and Schrader M. The peroxisome: an update on mysteries. Histochemistry and cell biology 2012;137:547–574.

20. Brocard C and Hartig A. Peroxisome targeting signal 1: Is it really a simple tripeptide? Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 2006; 1763:1565–1573.

21. Kamoshita M, Kumar R, Anteghini M et al. Insights Into the Peroxisomal Protein Inventory of Zebrafish. Frontiers in Physiology 2022;13. ISSN 1664-042X.

22. Morgat A, Lombardot T, Coudert E et al. Enzyme annotation in UniProtKB using Rhea. Bioinformatics 2019;36:1896–1901.

23. Li W and Godzik A. Cd-hit: a fast program for clustering and comparing large sets of protein or nucleotide sequences. Bioinformatics 2006;22:1658–1659.