neXt-MP50 and neXt-CP50 Challenge Updates based on Reports Received by May 11, 2019

Chromosome Number: 2

PIC Leaders: Lydie Lane

Major lab members or partners contributing to the neXt-MP50 Challenge

PaulaDuek (SIB/University of Geneva)
Alain Gateau (SIB/University of Geneva)
Thibault Robin (SIB/University of Geneva)
Frédérique Lisacek (SIB/University of Geneva)
Markus Mueller (SIB Lausanne)
Amos Bairoch (SIB/University of Geneva)
Charlotte Macron (Nestlé Institute of Health Sciences)
Antonio Nunez-Galindo (Nestlé Institute of Health Sciences)
Loïc Dayon (Nestlé Institute of Health Sciences)

Status of the Chromosome “parts list” for your Chromosome:
(https://www.nextprot.org/about/protein-existence)

There were 130 MP on chr 2 in 2017, now only 90 (71 PE2, 18 PE3 and 1 PE4).

A) Titles and authors of papers submitted to the 2019 JPR SI or planned.

neXt-MP50 Challenge


neXt-CP50 Challenge


B) Titles and authors of papers published in the 2018 JPR SI.

**neXt-MP50 Challenge**

*Large-Scale Reanalysis of Publicly Available HeLa Cell Proteomics Data in the Context of the Human Proteome Project.* Robin T, Bairoch A, Müller M, Lisacek F, Lane L.

*Identification of Missing Proteins in Normal Human Cerebrospinal Fluid.* Macron C, Lane L, Núñez Galindo A, Dayon L.

*Deep Dive on the Proteome of Human Cerebrospinal Fluid: A Valuable Data Resource for Biomarker Discovery and Missing Protein Identification.* Macron C, Lane L, Núñez Galindo A, Dayon L.

*Progress on Identifying and Characterizing the Human Proteome: 2018 Metrics from the HUPO Human Proteome Project.* Omenn GS, Lane L, Overall CM, Corrales FJ, Schwenk JM, Paik YK, Van Eyk JE, Liu S, Snyder M, Baker MS, Deutsch EW.

**neXt-CP50 Challenge**

*Exploring the Uncharacterized Human Proteome Using neXtProt.* Duek P, Gateau A, Bairoch A, Lane L.


**Other HPP projects**

*Update of the Functional Mitochondrial Human Proteome Network.* Monti C, Lane L, Fasano M, Alberio T.

*Toward Completion of the Human Proteome Parts List: Progress Uncovering Proteins That Are Missing or Have Unknown Function and Developing Analytical Methods.* Paik YK, Overall CM, Corrales F, Deutsch EW, Lane L, Omenn GS.

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.

N/A

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?

In Carapito et al. 2017, we reported the validation of 12 PE2 proteins by SRM and IHC.

In Robin et al. 2018, we reported the validation of 1 PE2 protein (FRAT2) by reanalysing MS/MS data on 41 HeLa cell datasets.

In the two articles by Macron et al. 2018, we reported the validation of 14 PE2 proteins and 1 PE5 protein (SHISA8) by analysing CSF by MS/MS.

E) How many PE1-found MPs since HUPO-2018 are now in NeXtProt as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.

Due to an incomplete processing of SRM data by PeptideAtlas, only 3/12 proteins validated by Carapito et al. 2017 are now PE1 in neXtProt.

12/16 of the other MPs validated by shotgun approaches are now PE1 in neXtProt.
A total of 15 proteins found by our team since 2017 are now PE1 in neXtProt (5 on chr 2)

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F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

Too many to be listed here
Chromosome Number: 3

PIC Leaders:
Takeshi Kawamura (Associate professor, Isotope Science Center, The University of Tokyo)

Major lab members or partners contributing to the neXt-MP50 Challenge

Lab member
Kazuki Yamamoto (Assistant professor)
Yoko Chikaoka (Researcher)
Sujin Lee (Researcher)

Co-PI
Toshihide Nishimur (Professor, St. Marianna University School of Medicine)
Hiromasa Tojo (Professor, St. Marianna University School of Medicine)

Status of the Chromosome “parts list” for your Chromosome:

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.

Manuscript in preparation.

A simple method for selecting a representative protein with better protein evidence from a set of non-unique proteins in outputs of Percolator Fido.
Hiromasa Tojo, Kiyonaga Fujii, Harubumi Kato, Toshihide Nishimura, Takeshi Kawamura

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.

Our group didn’t find PE1, but PE1 has increased nine from 960 to 969.

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

We have found four new proteins coded on chromosomes 1, 9, 17, and 20. However, only one peptide was detected, it doesn’t satisfy the requirement of the C-HPP Data Interpretation Guidelines. We have not found any for chromosome 3.
Chromosome Number: 4

PIC Leaders:
Yu-Ju Chen, Chia-Li Han, Ting-Yi Sung, Sung-Liang Yu

Major lab members or partners contributing to the neXt-MP50 Challenge
Mehari Muuz Weldemariam, Reta Birhanu Kitata, Wai-Kok Choong Yu-Chang Tyan

Status of the Chromosome “parts list” for your Chromosome: using January 11, 2019 neXtProt
https://www.nextprot.org/about/protein-existence

A) Titles and authors of papers submitted to the 2019 JPR SI or planned.


B) Titles and authors of papers published in the 2018 JPR SI.


C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.


D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?

26 PE1 MPs were reported in 2018

E) How many PE1-found MPs since HUPO-2018 are now in NeXtProt as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.

22 PE1-found MPs were promoted to PE1 level in neXtProt January 11, 2019 version.

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

A total of 87 Silver MPs were identified in the hESC dataset. Among these proteins, 4 MPs are located in
chromosome 4. The details are summarized in the following table.

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</table>
G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report.

Human embryonic stem cells (hESCs), a fundamental cell type, have unique features of cell renewal and pluripotency. Despite the significant progress of hESCs study, the complete hESC proteome atlas, especially the composition of cell surface factors responsible for maintaining the pluripotent nature of hESCs, are still under-explored. To our knowledge, this dataset covers the deepest membrane proteome profiles in hESC studies compared to previous literatures on the membrane proteomic studies on hESCs. Our deep subcellular proteomic profiles of hESC enabled us to map almost every molecule in the key pathways and network related to the

C-HPP 2019-05-11
regulation of hESCs.

(1) Based on the mRNA expression data in HPA, the identified gold and silver MPs were involved in different lineage specific differentiations, suggesting their potential functional roles in developmental biology. Many developmental proteins were identified in each lineage. For example, three gold MPs, SLC10A3, TREM251, and TREM37, were involved in the lung development.

(2) Many of them are involved in regulation of differentiation, self-renewal, epigenetic regulators, and cellular layers development through the core transcriptional networks in hESC. Interestingly, some identified MPs were associated with canonical pathways such as TGF-ß signaling (PCDHGA10 Gold MP, FRAT2-Silver MP) and MAPK signaling pathways (FRAT2-Silver MP, MMD-Silver MP), indicating their potential regulatory functions in hESC biology. Overexpression of FUT10 (Gold MP) has been reported to play a critical role in enhancement of self-renewal characteristics in embryonic stem cells [J. Biol. Chem. 2013, 288, 1076, 28859−28868]. In our data, this protein was confidently identified in more than one fractions (membrane and nucleus) and localized to Golgi membrane protein which was associated with STAT3 complex in LIF-AKT-STAT3 pathway. Thus, it may involve in regulating the core transcriptional networks by interacting with the key transcriptional factors including NANOG, SOX2 and OCT4 and eventually modulate self-renewal or cellular developments. G protein-coupled receptor 19 (GPR19, Gold MP), was reported among the list of 20 most positively significant genes in embryonic stem cells which may be crucial for maintaining or differentiating hESCs [Stem Cell Reports 2014, 10 (4), 472−479]. This Gold MP was identified in membrane fractions, consistent with its functional annotation as plasma protein receptor. Its role to regulate the GPCR signaling pathway and subsequently affect self-renewal or differentiation of hESCs remains further study.

Chromosome Number: 5

PIC Leaders: Peter Horovitch

Major lab members or partners contributing to the neXt-MP50 Challenge

Status of the Chromosome “parts list” for your Chromosome:

A) Titles and authors of papers submitted to the 2019 JPR SI or planned. Unfortunately, our team has no plan to submit paper in this coming issue. However, we have started to test a new tool that predict protein function using public data RNAseq, which may endup as a publication in the next issue (2020).
B) Titles and authors of papers published in the 2018 JPR SI.
None.

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.
in 2019

in 2018

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?
None (need more time to check all).

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.
None (need more time to check all).

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).
None (need more time to check all).

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report.
We have generated and shared via proteomeXchange several human proteomics datasets such as
- PXD013415, Quantification of surfactant protein D (SPD) in human serum by liquid chromatography-mass
spectrometry (LC-MS)

- PXD010115, Quantification of the Soluble Receptor of Advanced Glycation End-Products (sRAGE) by LC-MS Without the Use of Affinity Enrichment
- PXD008493, Assessment of sample preparation bias in mass spectrometry-based proteomics

### Chromosome Number: 6

**PIC Leaders:** Robert Moritz, Chris Overall,

**Major lab members or partners contributing to the neXt-MP50 Challenge**

Eric Deutsch, Frank Schmidt and John Wilson

**Status of the Chromosome “parts list” for your Chromosome:**

![Chromosome parts list](image)

A) **Titles and authors of papers submitted to the 2019 JPR SI or planned.**


B) **Titles and authors of papers published in the 2018 JPR SI.**


3. Paik, Y.-K., Overall, C.M., Corrales, F., Deutsch, E., Lane, L., Omenn, G.S. 2018. Toward Completion of the Human Proteome Parts List: Progress Uncovering Proteins that are Missing or have Unknown Function and Developing Analytical Methods. Journal of Proteome Research 17, 4023 – 4030.

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.


D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers? 0

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI. 0

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report.

A molecular corrector was developed that replaces the indole side chain of the mutant W->S in MALT1 that stabilised the protease and thus increased and rescued NFkB signalling.

**Chromosome Number:** 7

**PIC Leader;** Prof Ed. Nice

**Major lab members or partners contributing to the neXt-MP50 Challenge**

See later comments

**Status of the Chromosome “parts list” for your Chromosome:**
A) Titles and authors of papers submitted to the 2019 JPR SI or planned. N/A

B) Titles and authors of papers published in the 2018 JPR SI. N/A

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.


D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers? 0

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report. Analysis has shown that Chr 7, using our approach, continues to populate at a similar rate to other chromosomes

**Chromosome Number: 9**

**PIC Leaders:** Je-Yoel Cho

**Major lab members or partners contributing to the neXt-MP50 Challenge**
Soo-Youn Lee, Yong-In Kim, Dong Wook Kim, HuiSu Kim, Hyoung-Min Park, Jinwhan Eugene Lee

**Status of the Chromosome “parts list” for your Chromosome:**
A) Titles and authors of papers submitted to the 2019 JPR SI or planned.

B) Titles and authors of papers published in the 2018 JPR SI.
Launching the C-HPP neXt-CP50 Pilot Project for Functional Characterization of Identified Proteins with No Known Function.

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.
Gel-based proteomics in disease research: Is it still valuable?
Kim YI, Cho JY.

Quiescin Sulfhydryl Oxidase 1 (QSOX1) Secreted by Lung Cancer Cells Promotes Cancer Metastasis.

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?
We found DEFB123 with two unique peptides in a testis, which collected from maturation arrest (MA) patient, in 2017. However, DEFB123 was claimed by Wang et al., JPR, 2017 slightly earlier than our identification. Interestingly Wang et al. also find this MP in testis tissue.

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.
Not identified yet.

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).
We identified two peptides (13 AAs, 20 AAs) in a MA testis that only assigned to RGPD1 and RGPD2. These two MPs are highly similar, which differ in only one amino acid sequence.
G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report. We are trying to reveal biological function of MPs using human cell line models that express MPs and IP-MS. This strategy is not only useful for neXt-CP50 uPE1 functional characterization project, but also next-MP50 MPs identification and validation project. Two MPs (FOXD4, ARID3C) have been turned out its subcellular localization and binding partner proteins via our IP-MS strategy.

Chromosome Number: 10

PIC Leaders:
PI: Josh LaBaer
Co-I: Jin Park

Major lab members or partners contributing to the neXt-MP50 Challenge
Vel Murugan, Joe Miceli

Status of the Chromosome “parts list” for your Chromosome:

A) Titles and authors of papers submitted to the 2019 JPR SI or planned.
NA

B) Titles and authors of papers published in the 2018 JPR SI.
NA

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.
NA

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?
0

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.
5

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).
0

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report.
As a member of the 5-chromosome consortium of Chr 5, 10, 15, 16, and 19, we have been providing
the IVTT-compatible plasmids for missing proteins to other members for IVTT-assisted SRM and continue to generate more plasmids. We have assembled a comprehensive and one of the world’s largest collections of full-length Gateway plasmids representing more than 80% of all human protein-coding genes and are distributing the collection through our repository and distribution web portal DNASU (dnasu.org). Currently, we have full-length plasmids for around 62% of ~2,700 missing proteins (shown below) and aim to reach >90% by the end of 2019, which is available to the entire C-HPP team.

Chromosome Number: 11

PIC Leaders:
Dr. Jong Shin Yoo. (KBSI)

Major lab members or partners contributing to the neXt-MP50 Challenge
Jin Young Kim (KBSI), Bong Hee Lee (Gacheon Univ.), Heeyoun Hwang (KBSI)

Status of the Chromosome “parts list” for your Chromosome:
A) Titles and authors of papers submitted to the 2019 JPR SI or planned.

1. SAAVpedia: identification, functional annotation, and retrieval of single amino acid variants for proteogenomic interpretation, Soo Youn Lee, Heeyoun Hwang, Young Mook Kang, Hye Jin Kim, Ji Eun Jeong, Jin Young Kim, Jong Shin Yoo

B) Titles and authors of papers published in the 2018 JPR SI.

1. Identification of Missing Proteins in Human Olfactory Epithelial Tissue by Liquid Chromatography-Tandem Mass Spectrometry, Heeyoun Hwang, Ji Eun Jeong, Hyun Kyoung Lee, Ki Na Yun, Hyun Joo An, Bonghee Lee, Young-Ki Paik, Tae Seok Jeong, Gi Taek Yee, Jin Young Kim, and Jong Shin Yoo

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.

The PE level status of 11 Proteins in chromosome 11 were changed into PE1_2019 from PE2_2018. A list of them is shown as followed.

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<tr>
<th>Protein name</th>
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<th>Peptide Count (DP) in neXtProt_2019</th>
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Proteome Res.

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers? A total of 7 MPs was reported in our Chr. Group.

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<td>EFLQYSEGTLRS</td>
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</table>

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI. All 7 MPs were changed their status to PE1 level in now in neXtProt.

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in). N/A

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report. For the breakthrough to find out the MP 50, we need to analyse special human samples (e.g. Olfactory Epithelial Tissues) and develop an analytical method (e.g. membrane protein extracting method).
Chromosome Number: 12

PIC Leaders:
Ravi Sirdeshmukh

Major lab members or partners contributing to the neXt-MP50 Challenge
Hari PS, Mahesh Kulkarni, Srikanth Rapole, Sanjeev Shukla

Status of the Chromosome “parts list” for your Chromosome:

A) Titles and authors of papers submitted to the 2019 JPR SI or planned.
Not planned as yet.

B) Titles and authors of papers published in the 2018 JPR SI.
Nil.

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.
Jayaram S, Balakrishnan L, Singh M, Zabihi A, Ganesh RA, Mangalaparthi KK, Sonpatki P, Gupta MK,

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?

Not after 2017. We provided first MS evidence for 89 MPs in 2014. Out of which 60 have been entered in neXtProt. 29 are still listed among the MPs, as per recent version.

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.

Pl see ‘D’

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

29 out of 89 reported. Details not available. Filtered out probably on account of spectral quality.

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report.

Chromosome Number: 13

PIC Leaders: Young-Ki Paik
Major lab members or partners contributing to the neXt50:
Seul-Ki Jeong (Ph. D., YPRC)
Ju-Wan Kim (Ph.D., YPRC)
Jin-Young Cho (Ph. D., YPRC)
Keun Na (Ph. D., YPRC)
Chae-Yeon Kim (Ph. D candidate)

A) Titles and authors of papers submitted to the 2019 JPR SI or planned.

Progress on Identifying and Characterizing the Human Proteome: 2018-2019 Metrics from the HUPO Human Proteome Project. Omenn GS et al., (in preparation)
B) Titles and authors of papers submitted to the 2018 JPR SI and their status (e.g. in press, or under revision).

1. “ASV-ID, a Proteogenomic Workflow to Predict the Candidate Protein Isoforms based on Transcript Evidence.” Jeong SK, Kim CY, Paik YK (Published)


C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.

1. FusionPro, a versatile proteogenomic tool for identification of novel fusion transcripts and their potential translation products in cells (MCP, in revision)


D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?

0 protein (see F entry below)
E) How many PE1-found MPs since HUPO-2017 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.

4 proteins (NX_Q8NE28-1, NX_Q9H2F9-1, NX_C9J6K1-1 and NX_Q8N9B8) are now PE1 but we did not claim them as candidate MPs because they were one-hit wonders in our studies. (see F entry below).

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

12 proteins were found as candidate MPs but not fulfill the required numbers of peptides

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<th>Protein ID</th>
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<td>Not satisfied</td>
<td>One-hit wonder 2 2 Cho JY et al., 2017</td>
</tr>
<tr>
<td>NX_Q8N9B8</td>
<td>Not satisfied</td>
<td>One-hit wonder 2 1 Kim JW et al., 2016</td>
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</tbody>
</table>

Chromosome Number: 14

PIC Leaders:
Charles Pineau
Co-leader: Yves Vandenbrouck (BIG, BGE lab, CEA Grenoble)

Major lab members or partners contributing to the neXt-MP50 Challenge
- Nathalie Melaine (Protim, Inserm, University of Rennes)
- Christine Carapito (LSMBO, University of Strasbourg)
- Charlotte Macron (Presently at: Nestlé Institute of Health Sciences)
- Emmanuelle Com (Protim, Inserm, University of Rennes)
- Thomas Fréour (Reproductive Medicine unit, Nantes Hospital)
- Ongoing collaboration with Cecilia Lindskog and her group (HPA, Uppsala, Sweden)

Status of the Chromosome “parts list” for your Chromosome:

![Chromosome diagram](image)
A) Titles and authors of papers submitted to the 2019 JPR SI or planned.


B) Titles and authors of papers published in the 2018 JPR SI.

Related to the uPE1 challenge:

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.

N/A

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?

In Carapito et al., 2017, we reported the validation of 12 PE2 proteins by LC-SRM and IHC. Due to an incomplete processing of SRM data in PeptideAtlas, only 4 of them were upgraded to PE1 in neXtProt in 2018 (2 on chr 2 and 2 on chr 14).

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.

No new data since 2018

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

No new data since 2018

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report.

2 dark proteins characterized in the human testis (Melaine et al., 2018 JPR). Ongoing characterization of other candidates.

Chromosome Number: 15

PIC Fabio CS Nogueira and Gilberto B Domont

Major lab members or partners contributing to the neXt-MP50 Challenge

Gabriel RA Carneiro, Natália P Almeida, Clarissa F Mendonça, Erika Velasquez

Status of the Chromosome “parts list” for your Chromosome:

NO UPDATE
A) Titles and authors of papers submitted to the 2019 JPR SI or planned.

Planned:

Quantitative organellar proteomics of the orbitofrontal cortex of schizophrenia patients
Erika Velásquez, Ingrid Velásquez, Gabriel Reis Alves Carneiro, Andrea Schmitt, Peter Falkai, Daniel Martins-de-Souza, Gilberto B Domont, and Fabio CS Nogueira

and/or

Modelling premature cardiac aging by induced pluripotent stem cell from a patient with Hutchinson-Gilford Progeria Syndrome

B) Titles and authors of papers published in the 2018 JPR SI.

Launching the C-HPP neXt-CP50 Pilot Project for Functional Characterization of Identified Proteins with No Known Function

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.

Computational fluid dynamic analysis of physical forces playing a role in brain organoid cultures in two different multiplex platforms.

Mass spectrometry evaluation of a neuroblastoma SH-SY5Y cell culture protocol.
Muirillo JR, Pla I, Goto-Silva L, Nogueira FCS, Domont GB, Perez-Riverol Y, Sánchez A, Junqueira M.

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?

Candidates, only. See F below.

A large number of missing proteins of chromosome 15 is found in brain, thyroid and testis tissues. In the last three
years we concentrated our efforts in the proteomics of brain (normal and microcephalic ZIKV fetuses and stillborns-manuscript under review; cell and organellar proteomics of synapsis [published, SI2017], mitochondria, nuclei and cytoplasm of controls and schizophrenia adult patients [to be submitted, SI2019]); brain organoids [published]; brain cancer [published]; Hutchinson-Gilford Progeria Syndrome [ready for submission]; thyroid lobular controls and papillary thyroid carcinoma (final data collection).

Shotgun proteomics performed in high resolution, high sensitivity mass spectrometers was not able to find missing proteins that fulfilled the HPP Guidelines. We are reassessing this huge amount of data to look for any possible not-so-good-MS-spectra to validate by target-proteomics.

We are working on the development of new proteomics approaches to approach the missing protein problem. It is clear and obvious they are needed to solve the missing proteins challenge.

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.

In spite of a lot of work, NONE

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

1. Q9P2D8-3 Protein unc-79 homolog isoform Iso 3
Initial evidence at the transcript level, located in chromosome 14, two unique peptides of 11 and 10 amino acids

[R].RVSVASDPGRR.[V]
[K].NLPAGGAMIR.[C]

2. P0C7P4-1 Putative cytochrome b-c1 complex subunit Rieske-like protein 1 isoform Iso 1
Classified as uncertain, chromosome 22, one unique peptide of 14 amino acids

[K].EIKQEAVALSQLR.[D]

Chromosome Number: 16

PIC Leaders:

FJ Corrales team. Functional Proteomics Laboratory, CNB-CSIC, Madrid, Spain

Major lab members or partners contributing to the neXt-MP50 Challenge

Status of the Chromosome “parts list” for your Chromosome:
A) Titles and authors of papers submitted to the 2019 JPR SI or planned.
Two works ongoing but lack MPs validation

B) Titles and authors of papers published in the 2018 JPR SI.

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.
Liver cancer-associated changes to the proteome: what deserves clinical focus?

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?
NX_Q92839 Hyaluronan synthase 1

E) How many PE1-found MPs since HUPO-2018 are now in NeXtProt as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.
NX_Q92839 Hyaluronan synthase 1

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).
Q8TAV4. IPVQLQQR and VLAEGEMNASK. Found in 3 human thrombus. We were validating the observations but the protein has been annotated as PE1 in the last neXtProt release (2019)
NX_Q6PI77. We had 3 peptides in three independent cell lines that were under validation with synthetic peptides. The manuscript was going to be submitted to the 2019 SI but the protein has been already annotated as PE1.

We have identified the following candidates from PRIDE proteomics data from embryonic stem cells. Not all spectra look really reliable, the best candidate is NX_Q5TGS1. We are working to validate the data with SIL peptides.

NextProtID: NX_Q6IS14
        Instrument: Q-Exactive
        Publication url: http://europepmc.org/abstract/MED/28489815
        Pride Accession: PXD003903
        Cell type: IPS derived from fibroblasts

Peptide: RNDFQLIGIQDGYLSLLQDSGEVPEDLR
        score: 44.18
        Experiment-ID: PT-4825
        File: PT4825-13.raw
        Scan: 111446

Peptide: IVEMSASKTGK
NextProtID: NX_Q5TGS1
  Instrument: Q-Exactive
  Publication url: http://europepmc.org/abstract/MED/29489750
  Pride Accession: PXD005285
  Cell type: hPSCs

  Peptide: VWRPWGSPGDDLN
  score: 52.86
  File: 20130626_Q1_SA_NiHu_Mendjan_pluripotent_IgG_3.raw
  Scan: 32628

  Peptide: INVSLEQLK
  score: 63.50
  File: 20130626_Q1_SA_NiHu_Mendjan_pluripotent_IgG_3.raw
  Scan: 26816

NextProtID: NX_O95626
  Instrument: Q-Exactive
  Publication url: http://europepmc.org/abstract/MED/27233776
  Pride Accession: PXD002613
  Cell type: HEK-293

  Peptide: ASVGLEVLAEK
  score: 27.24
  File: MSA_5_6_03.raw
  Scan: 26011

  Peptide: CPNLIHLNLSGNK
  score: 70.37
  File: MSA_5_6_03.raw
  Scan: 20748

NextProtID: NX_AOAVI2
  Instrument: Q-Exactive
  Publication url: http://europepmc.org/abstract/MED/28489815
  Pride Accession: PXD003903
  Cell type: IPS derived from fibroblasts

  Peptide: LLLDIDNK
  score: 14.43
  Experiment-ID: PT-4711
  File: PT-4711-7.raw
  scan: 100800

  Peptide: RMAIEILAWGLR
  score: 41.80
  Experiment-ID: PT-4711
  File: PT-4711-3.raw
  Scan: 84104
NextProtID: NX_Q3KNW1  
Instrument: Q-Exactive  
Publication url: http://europepmc.org/abstract/MED/29489750  
Pride Accession: PXD005285  
Cell type: hPSCs  

Peptide: THTGEKPYACSHCSR  
score: 53.78  
File: 20130628_Q1_SA_NiHu_Mendjan_anterior_Smad2_2.raw  
scan: 4865  

Peptide: LLGAERMPR  
score: 44.55  
File: 20130628_Q1_SA_NiHu_Mendjan_anterior_Smad2_2.raw  
scan: 12981  

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report.

**Chromosome Number: 17**

**PIC Leaders:** Gilbert S. Omenn, Michael P. Snyder

**Major lab members or partners contributing to the neXt-MP50 Challenge**
Hongjiu Zhang, Omer Siddiqui, Chengxin Zhang, Yang Zhang

**Status of the Chromosome “parts list” for your Chromosome: Chromosome 17 has met the MP50 Challenge of documenting according to HPP Guidelines 50 MPs as PE1.**

A detailed analysis of the experimental and curation path to 43 MPs was published by Siddiqui et al, Chromosome 17 Missing Proteins: Recent Progress and Future Directions as part of the Next-50MP Challenge. J Proteome Res DOI: 10.1021/acs.jproteome.8b00442, 3 Oct 2018.

The extension to 50 MPs detected as now PE1 from Chr 17 was presented at US HUPO in March 2019 and is on the poster program for the 21st C-HPP Workshop in Saint-Malo in May 2019.

Please insert the figure for Chromosome 17. (I tried to copy and paste from neXtProt.)

Also, there seems to be no item in this form for uPE1 protein annotations. Wherever appropriate, please insert this sentence from C. Zhang et al, JPR, 2018: The I-TASSER/COFACTOR pipeline for structure-based function prediction was applied to all 66 uPE1 proteins coded on Chromosome 17; we inferred GO terms for biological activity with high confidence for 13 by MF, 33 by BP, and 49 by CC terms. Subsequently, we have performed a blinded prediction on 25 newly annotated neXtProt entries (none on Chr 17) in neXtProt release 2019-01, as will be presented at Saint-Malo C-HPP Workshop.
A) Titles and authors of papers submitted to the 2019 JPR SI or planned.
Planned manuscripts:
1. Zhang H, Guan Y, Omenn GS. Completion of the MP50 Challenge by Chromosome 17
3. Omenn GS and HPP leadership team: The Human Proteome as of 2019 based on Metrics from the HUPO Human Proteome Project.

B) Titles and authors of papers published in the 2018 JPR SI.
Toward Completion of the Human Proteome Parts List: Progress Uncovering Proteins That Are Missing or Have Unknown Function and Developing Analytical Methods. Paik YK, Overall CM, Corrales F, Deutsch EW, Lane L, Omenn GS. J Proteome Res. 2018 Dec 7;17(12):4023-4030. doi: 10.1021/acs.jproteome.8b00885. PMID: 30985145


C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.


Mapping genetic variations to three-dimensional protein structures to enhance variant interpretation: a proposed


D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers? 50 from Chromosome 17, meeting the MP50 Challenge. PE2,3,4 MPs reduced from 148 to 98.

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.
   7. PE2,3,4 MPs reduced from 105 to 98.

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).
   None. We stick to HPP Guidelines 2.1.

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report.

No

Chromosome Number: 19

PIC Leaders:
Sergio Encarnación-Guevara
Contributing labs (Lab Heads named with affiliation):
Ariadna Ortega, Leopoldo Gómez, Jeovanis Gil, Ramiro Alonso, Orlando Morales. Laboratory of Proteomics Center for Genomic Sciences UNAM.
Julio Collado-Vides, Program of Computational Biology at Center for Genomic Sciences UNAM.
Fernando Minauro Sanmiguel, Center for Medical Research in Human Genetics
Alejandro García Carranca, National Cancer Institute
Osvaldo Resendis Antonio, National Institute of Genomic Medicine.
Guadalupe Ayala, National Institute of Public Health

Partners:

Status of the Chromosome:
The Mexican consortium of chromosome 19 have 23 months since began projects aimed at meeting the objectives of the global consortium.

Chromosome 19 encodes for 1434 genes and 93% have been corroborated at protein level. Despite being the second biggest human chromosome, it stands as the third with the highest number of MPs. The neXtProt release 2019-01 listed 143 missing proteins (MPs) on Chr 19, which are grouped in four categories according to their degree of confirmation: 104 are in PE2, 25 in PE3, and 2 in PE4 and 32 in PE5. More than 100 of CH19 MPs are Zinc finger proteins and approximately the 10% are olfactory receptors. The high level of homology among these groups, presents a practical problem for unambiguous detection.

To claim protein discovery by MS, at least two non-overlapping proteotypic peptides longer than 9 amino acids need to be observed. These should also be discarded if they can be accounted for as sequence variants or isobaric PTMs of abundant PE1 proteins. The majority of MS based proteomic experiments are done with trypsin digestion; as such, it is important to note that two of the MPs belonging to Chr 19, do not have two tryptic non-overlapping proteotypic peptides of length at least 9

**Targeted Identification of MP using MS**

We performed an in-depth analysis in cervical cancer cell lines using different fractionation/separation protocols along with different protein extraction procedures with two aims: to study of lysine acetylation stoichiometry in cancer cells, and more importantly, to identify the largest possible number of missing proteins belonging to chromosome 19.

In a first ongoing step, we decided to choose a strategy to characterize the lysine acetylation at proteome level. Three different cell lines were used in this analysis: HaCaT (non-cancerous immortalized human keratinocyte), SiHa (HPV-16 positive cervical cancer cell line) and CaLo (HPV-18 positive cervical cancer cell line).

The method involves efficient total protein extraction and isotopically labeled chemical acetylation of proteins in order to differentiate them from endogenous acetylation. We applied this strategy to the analysis of these three cell lines cultured with or without EX-527 (potent inhibitor of the lysine deacetylase SIRT1). This lysine deacetylase, has been linked to tumor development, then, its inhibition empowers a situation normally observed in cancer. In this study we identified more than 10,000 proteins including 17 MPs from CH19.

The mixtures of peptides were subjected to prefractionation by reverse phase chromatography previous to LC-MS/MS Analysis, fractions were pooled together in 5 final fractions before injection to a Dionex Ultimate 3000 RSLC nano UPLC system in-line coupled to a high-resolution Q-Exactive Quadrupole-Orbitrap mass spectrometer, the protein identification was performed using MaxQuant.
Bioinformatic approaches to MPs fishing

A Bioinformatics analysis was carried out to characterize the MPs of the Chromosome 19 according to their feasibility to be identified by MS experiments. We performed an analysis considering those peptides with potential to be identified by mass spectrometry, based on their m/z range, and using different strategies or enzymes appropriate to be used in large scale proteomics study. We compared the number of peptides and proteins that can be analyzed with normal trypsin digestion (hydrolyzing after Lysine and Arginine residues), the second was the strategy where at protein level all lysine residues are chemically acetylated. Trypsin is unable to hydrolyze the peptide bond after acetylated Lysine. In consequence the generated peptides with this strategy are delimited by Arginine residues; and the third uses Lys-C instead of trypsin, which hydrolyzes the peptide bond after Lysine residues. As expected the number of peptides generated with normal trypsin digestion is almost twice the number of peptides that can be analyze by the other strategies. However, the number of proteins is not significantly reduced.

With a reduction of more than 40% in the average of peptides per protein, we consider to be in better possibilities to identify less abundant proteins, as the case of MPs. Even when pre-fractionation steps are included, the number of peptides is overwhelming and those from most abundant proteins consume more time of the LC-MS/MS measurement. In this sense a reduction in the number of peptides by restricting the cleavage sites of the enzyme will be highly valuable to our purpose of identifying MPs in Chromosome 19.

To further understand the beneficial effect of lysine acetylation in our chances to identify MPs, we analysed the chemical properties of all peptides derived from the Chr19 MPs that remain undetected. After "in silico" digestion of MPs, all peptides were submitted to Thermo Fisher’s “Peptide Synthesis and Proteotypic Peptide Analyzing Tool” to obtain an estimation of their properties. All peptides were also submitted to "Peptide uniqueness checker tool" from neXtProt to determine how many of them will actually be usefull in protein discovery. Beyond the reduction in the total number of peptides generated by trypsin after lysine acetylation, it is significant to remark that the percentage of unique peptides longer than 8 amino acids (Figure 1) increases from 21.21 to 37.8%, almost doubling the probability of finding a suitable sequence fragment to claim protein detection. Although an increment in average peptide mass was expected, it has been interesting to find that acetylation causes this parameter to be more evenly distributed from 1 to 4KDa as shown in Figure 2. While regular tryptic peptides show a clear tendency towards lower molecular weights, the histogram shows that acetylated peptides have a relatively constant probability density of having a mass in the range mentioned above. Surprisingly, there was no significant change in isoelectric point distribution, although we would have expected that blocking the amino groups in lysine side chains would have shifted the distribution towards the acidic region. A slight increase in average peptide hydrophobicity was also predicted after lysine acetylation. This can easily be explained by the average increase in peptide length resulting from blocking the ability of trypsin to cut at lysine residues.

Finally, we took all PE2 sequences from the chromosome 19, and we align them against every PE1 sequence from every chromosome in the human genome using Clustal as algorithm. We use the alignments to make decision trees by maximum parsimony. Using this strategy we were able to make relations about the sequence of the missing proteins and the proteins already known.

This help us to infer some characteristics, like the function of the protein, their possible domains, and localization in tissues. Taking this into account we choose the proteins more related to missing proteins and put it into a list. We upload this list to DAVID to know about it tissue localization. We identify brain, mammary gland, uterus, testis, among others, as possible localization to find these missing proteins.

Following this approach, we identified
Figure 1. Length distribution of non redundant tryptic peptides from MPs with or without lysine acetylation and usability in missing protein detection claim. Black color represents the fraction of unique peptides with no less than 9 amino acids which can be used for MP sight claim. Red shows the fraction of peptides longer than 8 residues but shared with PE1 proteins. Green represents all the peptides shorter than 9 residues whether they are unique or not.

Figure 2. Molecular weight distribution of unique peptides with no less than 9 amino acids resulting from tryptic digestion of Chr19 MPs. Black represents the peptides without lysine acetylation and red shows the distribution for the peptides from acetylated proteins.

Mapping of proteins identified against the neXtProt database revealed 244 MPs; 174 PE2 with experimental evidence at the transcript level, 68 proteins inferred from homology (PE3), 2 protein predicted (PE4). In addition we confirmed the expression of 17 MPs (PE2–PE4) belonging Chromosome 19 (Figure 3).

Figure 3. Identified MPs distribution by chromosomes and biological material.

The previous study we have reviewed in more detail, since we consider the most fruitful and with strategies that we consider should be taken into account in subsequent studies that involve the identification of MPs.

To validate some missing proteins belong to the chromosome 19, 17 unique peptides were selected based on visual inspection of PSMs and synthesized. The peptides were mixed together and analyzed by LC–MS/MS (Q Exactive Plus, Thermo Fisher Scientific) to acquire higher energy collisional dissociation (HCD) fragmentation spectra for comparison with the initial spectra in the closest possible conditions. In the figure 4 we have a example of the our analyses to validate the missing proteins, we found a peptide from the MP zinc finger protein 135 endogenously acetylated in the K277 residue. You can see the MS/MS spectrum pair.
Zinc finger protein 135 (ZNF135) found acetylated endogenously!

Peptide from ZNF135 with 1 Lys chemically acetylated and 1 Lys endogenously acetylated

![Figure 4. The upper spectrum corresponds to the peptide from the protein ZNF135 (a MP from CH19). This peptide was identified with our strategy of labeling all lysine residues with an acetyl group carrying heavy isotopes (Acetyl-d3). The peptide contains two lysine residues, one of them was chemically acetylated (heavy isotopes) during the acetylation reaction. However, the second K residue was found with a normal acetyl group, indicating endogenous acetylation. The peptide was identified by means of MaxQuant and by manual inspection. In the spectrum the most intense y and b series are pointed. In order to validate the identification, we synthesized the peptide with both lysine residues acetylated with the normal isotope composition (lower spectrum). The MS/MS spectrum showed very similar fragmentation pattern. The y series y10, y12 and y13 as well as b6 are shifted 3 Da relative to the peptide identified in the study, which was acetylated in one of the residues with an Acetyl-d3 group. Both MS/MS spectra clearly show fragments corresponding to both lysine residues, in our opinion we have no doubts regarding the identification of the peptide from ZNF135 or the presence of the PTM in its second lysine residue of the peptide.

Following this step, targeted assays using this approach were developed on the same LC–MS/MS platform to target 17 peptides belonging to 11 MPs, as we expected 10 of the 11 missing proteins validated using synthetic peptides are Zinc-finger proteins, and also 10 of 11 were detected expressed in cell treated with Sir1 inhibitor, EX527. (Table 1).

Ongoing projects.
Fernando Minauro and his group, analyzing mitochondria and vesicles from glioblastome cancer cell lines, identified 9 MPs (2 in cell lines, 2 in mitochondria and 4 in vesicles) of which only 1 belongs to chromosome 19, Figure 2 and Table 1. This approach is being carried out, because more than 10 percent of missing proteins belonging to Chromosome 19 have been proposed as olfactory receptors, in consequence we believe the brains cells could be a good source of missing proteins.

MS/MS analysis of five umbilical cords, previously we separate artery, vein, and Wharton jelly, after protein extraction we pooled the proteins obtained from each condition and the MS/MS analysis was performed using three different protocols: (i) 1D SDS-PAGE separation (23 gel slices); (ii) protein digestion, and peptides analyzed...
by nano-LC with long gradient runs; and (iii) protein digestion, and peptides fractionated by high-pH reversed-phase (Hp-RP) chromatography. For all protocols, tryptic peptides were analyzed by high-resolution MS instruments (Q-Exactive). In this preliminary analysis Ariadna Ortega and her group identified 60 MPs, artery 16, Wharton jelly 37 and vein, of which, belonging to Chromosome 19 we have (artery 1, Wharton jelly 2 and Vein 1). Figure 2 and Table 1.

Using iTRAQ approach combined with high-resolution mass spectrometry, Guadalupe Ayala and colleagues, analyzed biopsies from three stages: chronic gastritis, intestinal metaplasia, and gastric adenocarcinoma, and identified 22 MPs, 2 of them belong to Chromosome 19, Figure 2 and Table 1.

Using breast cancer cell lines, Alejandro García Carranca and his group, identified 2 MPs, one of them belongs to chromosome 19. Figure 2 and Table 1.

<table>
<thead>
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<td>Mitochondrion</td>
<td>PPP phosphatase family</td>
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<td>Immunoglobulin superfamily</td>
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<td>Excreted</td>
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<td>Thiol-dependent hydrolysis of ester, thioester, amide, peptide and isopeptide bonds formed by the C-terminal Gly of ubiquitin</td>
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<td>Nucleus</td>
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<td>May be involved in transcriptional regulation</td>
</tr>
</tbody>
</table>

Table 1. List of MPs belonging to chromosome 19 identified by all the strategies mentioned in this report. 1 Breast cancer, 2 Cervical cancer, 3 Gastric cancer, 4 Glioblastoma, 5 Artery (umbilical cord), 6 Vein (umbilical cord), 7 Wharton jelly (umbilical cord)

Other projects in progress, in very early stages and even without results.
We are running experiments using proteasome’s inhibitors, because, most proteins are cleared from the cell, via degradation through the proteasome, MG-132 is a potent inhibitor of the proteasome, it is working blocking the proteolytic activity of the 26S proteasome complex. Treating cells with MG-132 momentarily increase the stability of short-lived proteins. It could be the case of missing proteins with evidence at transcript level.

Purification of proteins containing zinc finger domains using immobilized metal ion affinity chromatography, with Ramiro Alonso Bastida as main participant, from Center for Genomic Sciences, trying to identified Zinc finger proteins which constitute more than half of MPs of the chromosome 19.
Chromosome Number: X

PIC Leaders:
PI: Yasushi Ishihama
Co-PI: Tadashi Yamamoto

Major lab members or partners contributing to the neXt-MP50 Challenge
Amr Elguoshy Abdeldayem Shalaby (Niigata University)
Shujiro Okuda (Niigata University)
Shin Kawano (Database Center for Life Science, DBCLS)
Susumu Goto (Database Center for Life Science, DBCLS)
Masaki Matsumoto (Kyusyu University)
Norie Araki (Kumamoto University)

Status of the Chromosome “parts list” for your Chromosome:
(https://www.nextprot.org/about/protein-existence)

A) Titles and authors of papers submitted to the 2019 JPR SI or planned.
1) The jPOST re-analysis workflow for public proteomics data repository

2) Rescue of the stranded peptides of human missing proteins in the GMP proteome database by referring with SRMAAtlas database
Amr Elguoshy, Yoshitoshii Hirao, Bo Xu, Naohiko Kinoshita, Keiko Yamamoto, Toshiaki Mitsui, Tadashi Yamamoto and Chromosome X project team of JPrOS (chair: Y. Ishihama).

B) Titles and authors of papers published in the 2018 JPR SI.

C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.
(1) The jPOST environment: an integrated proteomics data repository and database.

(2) Proteome Profiling of Diabetic Mellitus Patient Urine for Discovery of Biomarkers by Comprehensive MS-Based Proteomics.
Hirao Y, Saito S, Fujinaka H, Miyazaki S, Xu B, Quadery AF, Elguoshy A, Yamamoto K, Yamamoto T.

D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers? 41 MPs and 1 UP with “Stranded peptides”

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</table>

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI. 29 Proteins out of 41 MPs are now PE=1 in NeXtProt 2019

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).
Uncertain.

G) Any significant clinical or other successes re a MP that you wish us to consider highlighting in the report.
- We are still looking for Missing proteins in the GPM Db and the “Stranded peptides” by adapting a new more strict matching definition between fragmentation profiles from the GPM Db and SRMAtlas.
- The jPOSTdb has now a series of tools for hunting missing proteins from new datasets deposited into
jPOSTrepo, a member of ProteomeXchange Consortium under the jPOST environment, as follows:

- **jPOST missing protein search:**
  1. Visualize the distribution of PE1-5 categories automatically for each dataset with chromosome information.
  2. Check the missing protein candidates using nextprot peptide uniqueness checker.

- **jPOST-iMPAQ T db search for MRM transition setting**
  1. Check whether the MPs are within iMPAQ T, MRM transition database.
  2. Check mRNA expression of the MPs.
  3. MRM assay for MP validation

- PE2-4 contents (%) for each dataset in jPOSTdb can be used for the criteria to transfer to PeptideAtlas re-analysis pipeline.

**Figure**  The jPOST workflow for hunting missing proteins

<table>
<thead>
<tr>
<th>Chromosome Number: Mitochondria</th>
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</table>

**PIC Leaders:**
Andrea Urbani, Mauro Fasano, Paola Roncada

**Major lab members or partners contributing to the neXt-MP50 Challenge**
Maurizio Ronci
Luisa Pieroni
Viviana Greco
A) Titles and authors of papers submitted to the 2019 JPR SI or planned.

Planned:
Exploring the Dark Proteome of Human Mitochondria by DDA & DIA mass spectrometry. Federica Marini et al.

B) Titles and authors of papers published in the 2018 JPR SI.


C) Titles and authors of other HPP relevant papers submitted elsewhere in 2018/2019.


D) How many PE1-found MPs since HUPO-2017 has your chromosome group reported in papers?

3

E) How many PE1-found MPs since HUPO-2018 are now in NeXt-Prot as PE1 proteins? Please check each of your MPs that you reported in the JPR SI.

3

F) How many candidate MPs found, but not meeting the guidelines 2.1? (Please state number of peptides identified, their length, and biological replicates found in).

5
neXt-CP50 Challenge Update at May 11, 2019 St Malo

Chromosome Number: 2

Major lab members or partners contributing to the neXt-CP50 Challenge

Paula Duek (SIB/University of Geneva)
Camille Mary (University of Geneva)
Amos Bairoch (SIB/University of Geneva)

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

<table>
<thead>
<tr>
<th>Acc. code</th>
<th>Gene name</th>
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Chromosome Number: 4

Major lab members or partners contributing to the neXt-CP50 Challenge

Mehari Muuz Weldemariam, Reta Birhanu Kitata, Yu-Chang Tyan

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

The following two genes were selected for further characterization

BEND4, a transcription factor, highly expressed in the brain cell derived from iPSCs at mRNA level (Nature Genetics, 2015, 47, 132–141) and genome-wide studies identified as significantly hypermethylated in lung cancer at mRNA level (Int J Cancer, 2017 Nov 15;141(10):2014-2029).

ADRA2C, a GPCR protein, associated with cardiovascular complications such as bradycardia (Wu et al. Pharmacological Research 2018, 129) and its genotype influences heart failure severity (Reddy et al. Pediatr Res, 2015, 77). Kim et al. also reported as association between common genetic variants of α2A-, α2B- and α2C-adrenoceptors and the risk of silent brain infarction. Kraemmer et al demonstrated as potential for developing clinical-genetic models to identify patients with Parkinson's disease at increased risk (Kraemmer et al. J Neurol Neurosurg Psychiatry 2016, 87). The possible role of this gene in colorectal cancer metastatic progression has also been highlighted (Lee et al. BMC Med Genomics 2013, 5).
Major lab members or partners contributing to the neXt-CP50 Challenge

Robert Moritz (ISB)  Project lead, technology, bioinformatics
Chris Overall (UBC)  co-Project lead, technology
Eric Deutsch (ISB)  Bioinformatics
Frank Schmidt (Weill Cornell Medicine – Qatar)  Technology
John Wilson (Protifi, LLC)  Technology

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

Major lab members or partners contributing to the neXt-CP50 Challenge

Ker labs in Australia using a B/D-HPP approach to identify disease related proteins

Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

As detailed in previous reports we are using in silico methods (e.g MissingProteinPedia) or resources such as Protein Atlas to find biological or other non MS data in the literature to support the existence of these proteins and likely sources. Thus, for example, mmd2 (Monocyte To Macrophage Differentiation Associated 2) (No, No, No) in your table might be found in brain or testis or GPR85/SREB2 also in brain. Such studies could then inform/direct other BD groups

Major lab members or partners contributing to the neXt-CP50 Challenge

Anasuya Pal, Chenxi Xu

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

We performed genome-wide CRISPR-based function genomics screen to identify mutations that can promote cancer progression, especially invasion, in breast epithelial cells expressing different mutant p53 proteins. From the screens, a few hundred hits were identified for 2 different p53 mutants, and we are currently down-selecting the top candidates, including several uPE1 proteins, for individual validation. We are aiming to submit the manuscript describing the screening results in combination with RNA-Seq and ChIP-Seq data within 2019.

We are also producing more full-length plasmid clones for uPE1 proteins for functional studies, and the current clone coverage is shown below.
Chromosome Number: 11

Major lab members or partners contributing to the neXt-CP50 Challenge

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

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<th>Protein ID</th>
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<td>Coiled-coil domain-containing protein 90B, mCCDC90B</td>
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<td>Q8N3X1</td>
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Recently, we have found 4 UPE1s in cholangiocarcinoma from proteogenomic study, and will further study about their function using biological model system (eg. PDX + CRSPR-Cas9 etc.).

Chromosome Number: 12

Major lab members or partners contributing to the neXt-CP50 Challenge

Ravi Sirdeshmukh, Hari PS, Mahesh Kulkarni, Srikanth Rapole, Sanjeev Shukla

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

We have and continue to characterize one of the novel splice variant of NCAM 1. The variant has insertion of a novel exon. The novel variant has been shown as a full-length protein. Homology based structure modelling of the protein shows that the novel exon contributes evolutionarily conserved “low complexity motif” with implications in protein-protein interactions.
Chromosome Number: 13

Major lab members or partners contributing to the neXt-CP50 Challenge

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

1. Exploring the Function of Dark Proteome Encoded by Chromosome 13 for Biomedical Applications: Na K. et al., (in preparation)

2. We are currently focusing on YPRC-DP1 which showed a potential oncogenic property in the preliminary results by using the CRISPR/cas9 genome editing. We will be able to know the exact function of this dark protein when the cellular assays on the edited gene of DP1 will be done. From the pilot study, we also learned a few lessons on the analysis of dark proteins (uPE1) with respect to bottlenecks and potential pitfalls, which can be shared with other colleagues.

Chromosome Number: 14

Major lab members or partners contributing to the neXt-CP50 Challenge

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

Chromosome Number: 15

Major lab members or partners contributing to the neXt-CP50 Challenge

Felipe da Veiga Leprovost, Research Investigator, Department of Pathology, University of Michigan, An Arbor 
Gabriel RA Carneiro, Natália P Almeida, Erika Velasquez, Proteomics Unit, UFRJ, Brazil

A) Please list the CP50 Challenge Proteins that your team is characterizing and briefly describe your teams progress made to date including any publications or planned papers in 2019.

NX_Q6EEV4, DNA-directed RNA polymerase II subunit M, isoforms 4/5, Gene POLR2M GRINL1A

NX_Q8N5C7, DTW domain-containing protein 1, Gene DTWD1 MDS009

Literature search, software mastering, rationales, ongoing function data collection.

Chromosome Number: 16

Major lab members or partners contributing to the neXt-CP50 Challenge

FJ Corrales team. Functional Proteomics Laboratory, CNB-CSIC, Madrid, Spain

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

We are currently investigating the role of APIP in liver cells biology. This work is done in collaboration with Lydie's group who annotated a role of this enzyme in the methionine salvage pathway. We have already
generated stable PLC and Huh7 APIP deficient cells and the effects on cell proliferation, sensitivity to stress challenges are currently in progress.

We are also interested in the following chr16 uPE1 proteins that will be studied in the near future

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Chromosome Number: X

Major lab members or partners contributing to the neXt-CP50 Challenge
Yasushi Ishihama, Tadashi Yamamoto and Chr-X/jPOST teams

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

We are preparing the workflow to characterize proteins with unknown functions together with Yu-Ju Chen and her Chr-4 team. We hope we can show “proof-of-principle” of our strategy soon.

Chromosome Number: MT

Major lab members or partners contributing to the neXt-CP50 Challenge

A) Please list the CP50 Challenge Proteins that your team is characterising and briefly describe your teams progress made to date including any publications or planned papers in 2019.

Exploring the Dark Proteome of Human Mitochondria by DDA & DIA mass spectrometry. Federica Marini* a,b, Luisa Pieroni c , Victor Corasolla Carregari a,b, Viviana Greco a,b Massimo Castagnola a & Andrea Urbani a,b

a) Institute of Biochemistry and Clinical Biochemistry, Università Cattolica del Sacro Cuore, Rome, Italy
b) Department of Laboratory Diagnostic and Infectious Diseases, Fondazione Policlinico Universitario Agostino Gemelli-IRCCS, Rome, Italy.
c) Proteomic and Metabonomic Laboratory, Fondazione Santa Lucia-IRCCS, Rome, Italy

Mitochondria (mt) are essential organelles for cell life¹. Biological cellular functions such as respiratory chain, ATP production, ROS balance, and lipid metabolism are carried out by mt proteins and complexes associated both to the inner (IM) and outer (OM) mt membrane. We developed an experimental workflow with the aim of extensively characterizing the mt membrane proteome. We isolated the mitochondria by sucrose gradient², from HeLa cells and we separated the mt OM, IM by mitoplast preparation³. Collected proteins are digested by 3 different enzymes (Trypsin, Glu-C and Chymotrypsin) and the peptide fractions were acquired both by ACQUITY MClass System (Waters&CO) coupled to a DIA high definition IMS Synapt G2-Si Mass spectrometer and by nanoLC (Ultimate 3000) coupled to an Orbitrap Elite (ThermoFisher) collecting DDA MS/MS data. After combining all the acquired spectra and performing a relevant bioinformatic analysis, we were able to identify ~ 1100 proteins which are structurally and functionally related to mt and its membranes. Moreover, by using the advanced neXtProt dataset
(NXQ_00022) for searching Dark Proteins we found out the presence of 17 mt Dark proteins (PE1) in our experimental database and among them, we initially selected to investigate the protein *Threonine Synthase Like 1* (Accession Number: NX_Q8IYQ7).

1 Alberio T. et al. 2017, *J.of Proteome Res.*
1 Paik Y. et al. 2019, *J.of Proteome Res.*