



STRUCTURE AND FUNCTION OF THE HUMAN RIBONUCLEOSOME
GRANT AGREEMENT

between

NOMISSTIFTUNG
Claridenstrasse 26
CH-8002 Zurich
Switzerland

(hereinafter called "Foundation")

and

Universitat Bern
Department Chemistry, Biochemistry and Pharamcy
Freiestrasse 3
CH-3012 Bern
Switzerland

(hereinafter called "UNIBE" the "Grantee")

(hereinafter together referred to as "Parties")



PREAMBLE

- 1) The Foundation is a non-profit foundation established in accordance with Swiss law.
- 2) The Grantee filed a grant request with the Foundation ("**Grant Request**") with respect to a project as defined in the Grant Request;
- 3) The Foundation reviewed the Grant Request submitted by the Principal Investigators, xxxxxxxx, UNIBE, xxx, ETH Zurich, Switzerland, xxxxxxxx, Masaryk University – CEITEC, Czech Republic and xxxxx, University of Toronto, Canada and received it favorably;
- 4) The Foundation decided to support the project and provide a Grant to xxxxxxxx, UNIBE, who will be responsible to transfer the relevant grant amounts to the co-Pis as specified in Annex 1.
- 5) In order to formalize the grant by the Foundation to the Grantee, the Parties would like to enter into this grant agreement ("**Agreement**");

Now, therefore, the Parties agree as follows:

1. Project

1.1 Project Title

The Grantee is planning a project with the title 'Structure and Function Of The Human Ribonucleosome' with the aim to investigate how primary RNA transcripts are organized in the cell nucleus and how this spatial organization governs mRNA maturation and regulates gene expression. The research proposal is further described in the Grant Request, a copy of which is attached hereto as Annex 1 ("**Grant Request**").

Included as Annex 2 ("Project Plan and Governance") is the Project Governance delineating the separate responsibilities of UNIBE), ETH ZOrich, CEITEC and University of Toronto.

1.2 Project Duration

The duration is fixed to two years, with an agreed Start Date 1st February, 2022 and an agreed End Date 31st January, 2024



2. Grant

2.1 Award

Based on the Grant Request and the description of the Project contained therein, the Foundation decided to award to the Grantee the following conditional grant on the terms and subject to the conditions of this Agreement ("Grant"):

Amount granted ("Tatai Grant Amount"): CHF 947'279

The Total Grant Amount is divided between the Grantees as follows:

University of Bern	CHF 282'000
ETH Zurich	CHF 271'247
CEITEC	CHF 167'232
University of Toronto	CHF 226'800

2.2 Payment

Payment of the Grant Amount ("Grant Payment") shall be made during a period of two years as of signing of this Agreement (subject to the receipt of annual technical and financial reports) in annual installments with the specified amount as described in Annex 1 (Grant Request). The first payment to be made on or before January 31st 2022.

Amounts in Swiss Franks - CHF

	Year 1	Year 2	Total
xxxxxx, ETH Zurich	133,507	137,740	271,247
xxxxxxx, UNIBE	142,500	139,500	282,000
xxxxxxx, CEITEC	83,616	83,616	167,232
xxxxxx. University of Toronto	115,500	111,300	226,800
TOTAL	475,123	472,156	947,279

Grant Payments shall be made solely to the UNIBE and only by wire transfer to a bank account with a reputable international bank (Bank Account"). Upon receipt of the Grant Payment, UNIBESHall wire transfer immediately to ETH Zurich, CEITEC and University of Toronto annual instalments with the specified amounts as set aut in the budget pian Annex 1 (Grant Request).



Bank name and address: xxxxxxxxxxxx SWIFT / BIC: xxxxxxxxxxxx

Account Number / IBAN: xxxxxxxx Account holder: Universitat

Bern

Account holder address: Hochschulstrasse 6, 3012 Bern

2.2.1 Condition Precedent

The payment of the Grant Amount or any part thereof is subject to the Grantee being in full compliance with the terms of this Agreement at the date of payment.

3. Information and Reporting

3.1 Information Duties

The Grantee shall inform the Foundation without undue delay in writing, about:

- a) any material changes to the Project compared to the description in the Grant Request occurring or becoming foreseeable;
- b) any material delays of the Project occurring or becoming foreseeable;
- c) any new findings/information/changes which may considerably influence the realization of the Project and its targets;
- d) any decision by a competent governmental or non-governmental authority or committee with respect to the Project that, if the decision were to be upheld, would seriously threaten the continuation of the Project;
- e) any initiation of litigation by a third party with respect to the Project that, if the claims of the claimant were to be upheld, would seriously threaten the continuation of the Project; and
- f) the end of the Project, for whatever reason.



3.2 General Reporting Duties

The Grantees will collaborate and prepare the reports and documentation outlined below. UNIBE agrees to compile and submit required reports to the Foundation. CEITEC, ETH Zurich and the University of Toronto agrees to provide UNIBE with all necessary and relevant information and/or documentation including, but not limited to, financial reports, fifteen (15) days prior to the due dates listed below. The Grantees agree to compile and submit required reports to the Foundation. The Grantees shall provide the Foundation with, and the Foundation shall have unrestricted access to, the following information:

- a) within 30 calendar days of the end of each calendar year, a reasonably detailed annual donor report informing about the progress of the Project and the use of the Grant, including the current accounts of the Project, using the templates provided by the Foundation;
- b) within three months of the end, for whatever reason, of the Project, a reasonably detailed final donor report informing about the realization of the Project, the target achievements and the use of the Grant, including the final accounts of the Project;
- c) forthwith , without undue delay, a copy of any decision by a competent governmental or non-governmental authority or committee with respect to the authorization of the Project;
- d) forthwith , without undue delay, a copy of any update approval letter or similar documents provided by or to a competent governmental or non-governmental authority or committee with respect to the authorization of the Project; and
- e) forthwith, without undue delay, any additional information or documents reasonably requested by the Foundation in order to (i) account for the Grant made to the Grantee, or (ii) meet the demands of any regulatory or governmental authorities.
- f) forthwith the Grantee and the Foundation acknowledge and agree that Prof. xxxxxx together with xxxxxx, xxxxxx and xxxxxx to deliver at least one detailed update in person on the progress and/or outcome of the research project to the Foundation Board of Directors, if requested, at a mutually acceptable time and date.

4. Participation in Events and Publications

The Grantee agrees, in principle, to delegate xxxxxxxx and other experts engaged in the Project, including the together with xxxxxxxx, xxxxxxxx and xxxxxxxx s, to events organized, hosted or sponsored by the Foundation (e.g. symposia , expert conferences or meetings, panel discussions) and to contribute to scientific publications sponsored or issued by the Foundation.



All reasonable efforts shall be made to properly recognize the NOMIS foundation in presentations, publications and other forms of communications.

Publications of the Grantee (including abstracts of presentations at scientific, clinical meetings, conferences and symposia) resulting from the Project must carry the following acknowledgment:

"This research project and related results were made possible with the support of the NOMIS Foundation."

5. Representations and Warranties / Guarantees

5.1 Representations and Warranties

The Grantee represents and warrants that at signing of this Agreement:

- a) the content of the Grant Request and in particular the description of the Project contained therein is true and accurate and not in any way misleading;
- b) the Grantee or the institution the Grantee is an employee or a subsidiary of is the sole account holder and beneficial owner of the Bank Account;
- c) to the extent any approval or permission of a governmental and non-governmental authority or committee is required to start or continue the Project according to applicable law (in particular including any approval for testing involving human subjects, tissues or cells, including embryonic stem cells, or vertebrate animals), such approval or permission has been granted to the Grantee.

5.2 Guarantees

The Grantee guarantees that:

- a) the Grant Amount is used exclusively for the Project;
- b) the Grantee remains the sole account holder and beneficial owner of the Bank Account;
- c) the Grantee will comply with any local law applicable to the Project and it will pursue the Project in compliance with any conditions, reservations and requirements of any competent governmental or non-governmental authority or committee;
- d) the Project is not and will not be primarily aimed at monetary profit or securing intellectual property rights or any commercialization of the Project results;
- e) the results of the Project will be made available to the public on an ongoing basis and free of charge; and
- f) the Project and the Grant will not be used for any kind of political, ideological or religious activism.



Managing Director

xxxxxxxxxx

If to the Grantee:

xxxxxxxxxx

Universitat Bern

xxxxxxxxxx

such other address as any of the Parties may notify to the other Party in accordance with the above.

8.3 Costs and Taxes

All costs and taxes relating to this Agreement shall be borne by the Parties, with each Party bearing its own costs and taxes.

8.4 Confidentiality and Public Relations

8.4.1 Confidentiality

Each Party shall keep the contents of this Agreement confidential. None of the Parties shall disclose or publicly announce the terms of this Agreement without the other Parties' prior written consent, except that no such prior written consent shall be necessary, if such Party is compelled to disclose such information by applicable law, court or governmental order or has to comply with applicable statutory reporting requirements.

8.4.2 Public Relations

Notwithstanding any confidentiality obligation, the Parties may disclose information about the Project including its progress, about the Grant and about the Parties' cooperation to the public as part of their public relations work.

The parties endeavor to mutually coordinate their public relations activities and will apply best efforts to agree on the manner and content of the communication activities related to the Project or the Grant. Therefore, each party undertakes to inform the other party about its planned public relations activities ahead of time in order to allow the other party to comment.



8.5 Entire Agreement

This Agreement, including the annex, constitutes the entire agreement and understanding between the Parties with respect to its subject matter and shall supersede all prior oral and written agreements or understandings between the Parties with respect to its subject matter.

8.6 Amendments and Waivers

Any amendment or addition to this Agreement has to be in writing and signed by both Parties.

No failure to exercise, nor any delay in exercising, on the part of any Party, any right or remedy under this Agreement shall operate as a waiver, nor shall any single or partial exercise of any right or remedy prevent any further or other exercise or the exercise of any other right or remedy.

8.7 Assignment

The Grantee may not assign this Agreement nor any rights or obligations hereunder to any third party without the prior written consent of the Foundation.

8.8 Severability

Should any part or provision of this Agreement be held to be invalid or unenforceable by any competent arbitral tribunal, court, governmental or administrative authority having jurisdiction, the other provisions of this Agreement shall nonetheless remain valid. In this case, the Parties shall negotiate in good faith a substitute provision that best reflects the economic intentions of the Parties without being unenforceable, and shall execute all agreements and documents required in this connection.

9. Governing Law and Arbitration

This Agreement shall be governed by and construed in accordance with the substantive laws of Switzerland.

The ordinary courts of the Canton of Zurich at Zurich 1 shall have exclusive jurisdiction for all disputes arising out of or in connection with this Agreement, including disputes on its conclusion, binding effect, amendment and termination.

[signatura page following]

XXXXXXXXXX





ANNEX 1- GRANT REQUEST

Appendix to Grant Application "Structure and Function of the Human Ribonucleosome"

Principal Investigators:

- xxxxxxxx, ETH Zurich, Switzerland
- xxxxxxxx, University of Bern, Switzerland
- xxxxxxxxxxxx, CEITEC, Brno, Czech Republic
- xxxxxxxx, University of Toronto, Canada

1.) Feedback from NOMIS after reviewing our grant proposal

After a discussion of NOMIS Board members with two external experts in the field, xxxxxxx gave us the following feedback on our grant proposal:

In the light of missing compelling data that the ribonucleosome indeed represents a biologically important entity of the cell, the proposed research plan was judged as too expansive and overambitious and the requested financial support as too high. As a result of this evaluation, we were asked to prioritize the proposed experiments and describe a proof-of-principle study with a reduced budget in which we focus on providing compelling evidence for the functional importance of the ribonucleosome and its existence in cells. Accordingly, it was suggested that this proof-of-concept phase should be reduced both in terms of the number of researchers involved (one postdoctoral fellow per lab) and duration (two years instead of three). If data resulting from the proof-of-concept study demonstrate the importance of the ribonucleosome for cell function, NOMIS would consider continued funding for further investigating the structure and function of the human ribonucleosome.

2.) Adjustment of the research plan and priority setting for the proof-of-concept phase by the four principal investigators

The applicants appreciate the feedback of NOMIS and the opportunity to define a proof-of-concept phase of their research plan. Based on the request to focus on providing functional data addressing the biological importance of the ribonucleosome and on the PI's strong desire to keep the collaboration between the four labs intact, priority will be given to Aim 3 of the initial proposal, complemented with selected experiments described in the original Aims 1 and 2. Aim 4 will not be addressed in the proof-of-concept phase.

In brief, we plan to conduct UV crosslinking followed by immunoprecipitation (CLIP) in cells and on biochemically purified 40S particles to determine the binding sites of hnRNP C and hnRNP A1 on the RNA (xxxxxx and xxxxxxxx labs). These data will be intersected with RNA-seq performed on the 40S-associated RNA. Collectively, this will provide a definitive transcriptome-wide map of the 40S binding sites and will allow us to perform a comparison of *in vivo* versus *in vitro* binding (xxxxxx and xxxxxxxx labs). A high overlap of the identified *in vitro* and *in vivo* binding sites would be a strong indication that the 40S particle indeed exists in cells and does not represent a purification artefact, which apparently was a concern of one external reviewer. In parallel, we will support our cross-linking experiments by visualization of the 40S particles inside nuclear extracts as well as inside the intact nuclei by cryo-electron microscopy imaging (xxxxxx and xxxxxxxx labs).

To provide evidence for the biological importance of ribonucleosomes, we will perform siRNA-

mediated knockdowns of hnRNP C and hnRNP A1 and isolate and deep-sequence the nuclear and cytoplasmic RNA of the cells to elucidate alterations on pre-mRNA splicing and nuclear export caused by disrupting ribonucleosomes (xxxx and xxxxx labs). We will initially focus on splicing and export, because our preliminary analysis already indicates that the ribonucleosome is involved in the regulation of these two processes. However, we will also look for additional perturbations and phenotypes. Depletion of individual hnRNP proteins does not however provide proof that observed functional phenotypes are due to a lack of ribonucleosome formation, as they could in principle also be the result of the loss of a function that the respective hnRNP protein exerts independently of its assembly into a 40S particle. Therefore, an important goal of our proof-of-concept study is to identify specific mutations in ribonucleosome components that prevent the formation of the 40S particles while still allowing the individual hnRNP proteins to bind RNA. The identification of amino acid residues of hnRNPs C and A1 that are required for 40S formation will be guided by CLIR-MS and NMR structural data revealing critical amino acid residues (xxxxx lab), and by making use of our *in vitro* reconstitution system, in which we can form 40S particles from recombinant hnRNP A1, hnRNP C and an *in vitro* synthesized RNA (xxxxx and xxxxx labs). If point mutations that prevent 40S formation *in vitro* can be identified, we will express the respective hnRNP protein(s) carrying such mutations in cells and determine their effects on pre-mRNA splicing and mRNA export (xxxxxx and xxxxx labs). In parallel, we will carry out structural analysis of the *in vitro* assembled mutant 40S particles using cryo-electron microscopy (xxxxxx lab).

We are convinced that with the above described approaches, we will be able to significantly advance our project during the next two years and provide compelling evidence for the biological importance of ribonucleosomes.

3.) Required budget for the proof-of-concept phase (calculated in Swiss Francs):

Budget xxxxxxxx (ETH Zürich, Switzerland)

	Year 1	Year 2	Total
Salary for Postdoc	107007	112240	219247
Consumables	17500	17500	35000
Facility fees	7000	6000	13000
Travel expenses	2000	2000	4000
Total sum requested	133507	137740	271247

xxxxxxx (Univ. of Bern, Switzerland)

	Year 1	Year 2	Total
Salary for Postdoc	110000	110000	220000
Consumables	17500	17500	35000
Facility fees	13000	10000	23000
Travel expenses	2000	2000	4000
Total sum requested	142500	139500	282000

Budget xxxxxx (CEITEC, Brno, Czech Republic)

	Year 1	Year 2	Total
Salary for Postdoc	44116	44116	88232
Consumables	17500	17500	35000
Facility fees	20000	20000	40000
Travel expenses	2000	2000	4000
Total sum requested	83616	83616	167232

Budget xxxxxxxxx (Univ. Toronto, Canada)

	Year 1	Year 2	Tatai
Salary for Postdoc	70000	72800	142800
Consumables	17500	17500	35000
Facility fees (NGS)	25000	18000	43000
Travel expenses	3000	3000	6000
Total sum requested	115500	111300	226800

BUDGET SUMMARY

	Year 1	Year 2	Tatai
Budget xxxxx	133507	137740	271247
Budget xxxxxx	142500	139500	282000
Budget xxxxxxxxx	83616	83616	167232
Budget xxxxxxxxxx	115500	111300	226800
Budget for entire project	475,123	472,156	947,279



ANNEX 2 - GOVERNANCE

xxxxxx is the designated contact person for NOMIS. He and his institution UniBE are responsible for ensuring through a Consortium Agreement signed before the Grant Agreement is signed or no later than Jan 31st 2022 by UniBE and the three other project partners that the terms stipulated in the Grant Agreement with NOMIS are fulfilled. The other three project partners xxxxxx, xxxxxx and xxxxxxxxxx and their respective institutions are responsible for supporting xxxxxxxx in managing the grant according to the Grant Agreement. The principal investigators of the partner labs are responsible for the scientific and scholarly aspects of the project.

The Consortium Agreement includes terms regarding:

- Reference to this Grant Agreement
- Decision making process
- Payments
- Access to and sharing of project results
- Scientific Publications

Scientific decisions (e.g. project design and changes in the research plan) will be discussed among and decided by the project partners. Unanimous decisions on direction will be attempted. If this cannot be achieved, the majority vote is the deciding vote. For new personnel hired on this project, the hiring project partner shall consult