DATED the day of 2015

BETWEEN

INSTITUTE OF BIOENGINEERING AND NANOTECHNOLOGY

AND

BIOINFORMATICS INSTITUTE

AND

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
NATIONAL CENTER FOR COMPUTATIONAL TOXICOLOGY

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AGREEMENT FOR RESEARCH COLLABORATION

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Ref: AH/OCL/667/0615/IBN

AGREEMENT FOR RESEARCH COLLABORATION

THIS AGREEMENT is made on the day of 2015

BETWEEN

BIOINFORMATICS INSTITUTE , BIOMEDICAL SCIENCES INSTITUTES, (Co. Reg. No. 199702109N) having its principal office at 30 Biopolis Street, Matrix #07-01 Singapore 138671 ("**BII**");

INSTITUTE OF BIOENGINEERING AND NANOTECHNOLOGY, BIOMEDICAL SCIENCES INSTITUTES, (Co. Reg. No. 199702109N) having its principal office at 31 Biopolis Way #04-01 The Nanos, Singapore 138669 ("**IBN**");

AND

The NATIONAL CENTER FOR COMPUTATIONAL TOXICOLOGY of the UNITED STATES ENVIRONMENT PROTECTION AGENCY, located in Research Triangle Park, NC, the UNITED STATES of AMERICA (hereinafter referred to as "**EPA" and "NCCT**").

(Each of BII, IBN, and EPA are hereinafter referred to individually as "a Party" and collectively as "the Parties." IBN and BII are hereinafter collectively referred to as "the A*STAR RIs.")

RECITALS

(A) The A*STAR RIs are national research institutions based in Singapore and funded by the Agency for Science, Technology and Research ("A*STAR").

(B) IBN has considerable knowledge, expertise and experience in, inter alia, the field of toxicology tissue models and assays.

(C) BII has considerable knowledge, expertise and experience in, inter alia, the field of computational biology.

(D) EPA is an Agency of the United States of America. NCCT is a Laboratory under the EPA. NCCT has an interest and expertise in, and proprietary technologies and know-how in the fields of computational toxicology.

(E) The A*STAR RIs and NCCT wish to collaborate in research and development in the areas of interest referred to above by undertaking the Project (as defined below) on the terms and conditions set out below.

NOW IT IS HEREBY AGREED as follows: -

1. DEFINITIONS

In this Agreement, unless otherwise expressly provided, the following terms shall have meanings ascribed to them below.

"**Affiliates**" means: (i) an organisation, which directly or indirectly controls either Party; or (ii) an organisation which is directly or indirectly controlled by either Party; or (iii) an organisation, which is controlled, directly or indirectly, by

the ultimate parent company of either Party. The term “control” as used herein means the possession of the power to direct or cause the direction of the management and the policies of an entity, whether through the ownership of a majority of the outstanding voting security or by contract or otherwise. The term ‘Affiliate’ with regards to the A*STAR RIs shall include A*STAR, ETPL and all research institutes and centres funded and managed by A*STAR.

“**A*STAR**” means the Agency for Science, Technology and Research.

[redacted]

"**Confidential Information**" means the terms of this Agreement and any and all information, data, designs, memoranda, models, prototypes, and/or other material whether of scientific, technical, commercial, financial or other nature, furnished to or obtained by a Party from the other Party under this Agreement in written, oral or other tangible form clearly marked or designated as “Confidential” or by words of similar import. Information communicated by a disclosing Party orally or visually shall be summarized in writing, marked “Confidential” and delivered to the receiving Party within [redacted] days of such communication, failing which such information shall not constitute Confidential Information.

“**Effective Date**” means the date of the last signature of the parties on this agreement.

[redacted]

“**ETPL**” means Exploit Technologies Pte Ltd, the commercialization and marketing arm of A*STAR and the A*STAR RIs.

[redacted]

“**Intellectual Property (IP)**” means all patents, Inventions, copyright, registered designs, and semiconductor layout designs in all countries of the world arising under statutory or common law, and whether or not perfected, and any pending applications of the foregoing.

"Invention" means any invention or discovery which is or may (or may not) be patentable or otherwise protectable under the intellectual property laws of this or any foreign country.

[redacted]

“**Pre-Collaboration IP**” means all IP owned or controlled by each Party and which was conceived or reduced to practice either (a) prior to commencement of the work performed pursuant to this Agreement or (b) outside the scope of the work performed pursuant to this Agreement and which is introduced to or disclosed for the Project or otherwise supplied by each Party; and for the A*STAR RIs shall mean the Pre-Collaboration IP which is expressly documented by it and made available to EPA.

“**Project**” means the research and development activities specified in the Project Plan.

"**Project IP**" means all IP [redacted] which was discovered, developed, conceived or reduced to practice by a Party or their employees, servants, invitees or agents in the course of the Project.

"**Project Plan**" means the statement of work set out in Schedule 1 annexed hereto.

[redacted]

[redacted]

"**Term**" means the period as specified in Clause 4.

[redacted]

2. STATEMENT OF WORK

2.1 The Parties hereby agree to collaborate in the Project.

2.2 The Parties recognize that the Project is research in nature and hence completion within the period of performance, or within the limits of financial support allocated, or the achievement of the deliverables and/or milestones specified in the Project within or outside the time schedule specified therein cannot be guaranteed. The Parties shall exercise reasonable efforts in the performance of the Agreement in accordance with the agreed scope of work.

2.3 The Parties agree and declare that the obligations of the Parties shall cease (except as otherwise set forth in Clause 13.2) upon the end of the Term.

2.4 Each Party shall obtain all relevant ethics and other approvals as may be relevant for its participation in the Project.

3. CO-ORDINATORS

3.1 The Project shall be supervised and coordinated by Daniele Zink from IBN, (hereinafter referred to as "IBN Co-ordinator"), Loo Lit Hsin from BII, (hereinafter referred to as "BII Co-ordinator") and Keith Houck from NCCT (hereinafter referred to as "NCCT Co-ordinator").

3.2 If for any reason an A*STAR RI Co-ordinator is unable to continue to serve under the Project, the A*STAR RI agrees to appoint a successor within [redacted] days of the unavailability of the Co-ordinator, failing which the provisions of Clause 12.2 shall apply.

4. PERIOD OF PERFORMANCE

This Agreement shall come into force on the Effective Date and shall continue for a period of three (3) years unless earlier terminated in accordance with the terms of this Agreement or extended by the Parties' agreement in writing.

5. PROJECT CONTRIBUTIONS

Each Party will make such contributions in terms of manpower deployment, equipment, facilities, cash funding and other contributions as specified in

Schedule 1, or as agreed from time to time by the Parties in writing.

6. PUBLICATIONS

6.1 Each Party may publish at any symposia, national, international or regional professional meeting or in any journal, thesis, dissertation, newspaper or otherwise of its own choosing, the findings, methods and results derived from the Project, but always subject to due observance of this Clause 6.

6.2 The Party intending to make the publication ("the Publishing Party") shall furnish the other Parties ("the Other Parties") copies of such proposed publication or presentation in advance of the submission of such proposed publication or presentation to a journal, editor, or other third party. The Other Parties shall within [redacted] days of receipt of the proposed publication or presentation forward its written objections to the same either because there is patentable subject matter that needs protection and/or there is Confidential Information (as defined in Clause 1 herein) or patentable information of the Other Parties contained in the proposed publication or presentation. If no objection is made to the proposed publication or presentation within the stipulated time, the Publishing Party shall be free to proceed with the publication or presentation.

6.3 Confidential Information identified by the Other Parties, which is governed by Clause 7, shall be deleted from the proposed publication or presentation unless the Other Parties considers the Confidential Information to be patentable information, in which case it will be treated as set forth in the following sub-Clause.

6.4 In the event that a Party objects to any such publication or presentation on the basis that the same would disclose patentable information belonging to that Party, the Publishing Party shall refrain from making such publication or presentation for a further period of [redacted] days from date of receipt of such objection in order for the relevant patent application(s) to be filed.

6.5 Each Party shall, in any publications it makes in relation to the methods, results and findings of the Project, acknowledge the other Party's contributions to the Project.

7. CONFIDENTIALITY

7.1 Each Party agrees, for the Term and for a period of [redacted] months after the termination or expiration of the Agreement, to treat the Confidential Information of the other Parties as strictly confidential and not to disclose it to any third party for any purpose whatsoever and not make use of the Confidential Information or any part thereof other than for the Project and to treat it with at least the same care and in the same manner as its own secret and valuable information. The receiving Party shall ensure that its employees to whom Confidential Information is disclosed covenant to keep such information confidential to the extent that the receiving Party is bound by this Agreement and that such covenants on the part of employees are strictly observed.

7.2 The provisions of Clause 7.1 above shall not apply to any:

7.2.1 information which is or was already known to the receiving Party at time of disclosure to it, or

7.2.2 information which after disclosure to the receiving Party under this Agreement is published or otherwise generally available to the public otherwise than through any act, default or omission by the receiving Party of its obligations hereunder, or

7.2.3 information which can be established by tangible evidence was independently developed by the receiving Party without the use of or reference to the disclosing Party's Confidential Information; or

7.2.4 information which is required to be disclosed to governmental or regulatory bodies or to a court of competent jurisdiction pursuant to any written law, provided, however, that such disclosure is limited to that required to be disclosed; or

7.2.5 information which, pursuant to a court order, is required to be disclosed as evidence in a court of law, provided however that such disclosure is limited to that required to be disclosed; or

7.2.6 information which is disclosed to the receiving Party by a third party without restriction and without breach of the confidentiality obligations under this Agreement by the receiving Party.

7.3 It is agreed that the A*STAR RIs may disclose all or any part of the Confidential Information to their Affiliates on the basis that the A*STAR RIs shall procure that such Affiliates shall also agree to treat the information as confidential.

7.4 The receiving Party acknowledges that unauthorized disclosure or use of Confidential Information could cause great or irreparable injury to disclosing Party and that pecuniary compensation would not afford adequate relief or it would be extremely difficult to ascertain the amount of compensation which would afford adequate relief. Therefore, the receiving Party agrees that, in the event of such unauthorized disclosure or use of Confidential Information, the disclosing Party will have the right to seek and obtain injunctive relief in addition to any other rights and remedies it may have.

7.5 Except for the disclosure of the existence of this Agreement, including the title and identification of the Parties, which information shall not be deemed confidential, no Party shall disclose the specific terms and conditions of this Agreement without the express permission of the other Parties or as required by applicable laws.

8. INTELLECTUAL PROPERTY, DATA AND RESULTS

8.1 The Parties do not anticipate the necessity for the use of any third party licences ("Third Party Licence(s)") for the conduct of their scope of work under this Agreement.

8.2 All rights, title and interests to Pre-Collaboration IP shall remain with the Party introducing or disclosing the same and shall remain unfettered by this Agreement. Each Party grants to the other Parties the right to use its Pre-

Collaboration IP for the purposes of the Project during the Term and for no other purposes except as provided in this Agreement.

[redacted]

9. COLLABORATION

For the avoidance of doubt, it is agreed that notwithstanding the terms and conditions of this Agreement, each Party will have the following rights:

(a) to conduct any research or development work in any field (including work relating to the research contemplated under this Agreement) independently of the other Party, whether by itself or in collaboration with any other party subject to each Party observing the provisions of Clause 7 hereof;

(b) to continue existing commitments or to make new ones; and

(c) to use, exploit (including sub-licensing) or otherwise take advantage of its own Intellectual Property [redacted].

10. WARRANTIES AND LIABILITIES

10.1 Each Party represents and warrants that it has the right to enter into this Agreement and provide the materials and services described herein. Except for the foregoing, the Parties do not make any representations, conditions or warranties, either express or implied with respect to any information, its Pre-Collaboration IP, the work performed pursuant to the terms of this Agreement, or the Project IP developed under this Agreement. Without limiting the generality of the foregoing, each Party expressly disclaims any implied warranty, condition or representation that the said information, its Pre-Collaboration IP and/or the Project IP developed under this Agreement:

10.1.1 shall correspond with a particular description;

10.1.2 is of a merchantable satisfactory quality;

10.1.3 is fit for a particular purpose; or

10.1.4 is durable for a reasonable period of time.

10.2 Nothing in this Agreement shall be construed as:

10.2.1 a warranty by any Party that anything made, used, sold or otherwise disposed of in connection with its Pre-Collaboration IP disclosed or introduced hereunder or that the Project IP or Project IP developed is or will be free from infringement of patents, copyrights, trademarks, industrial designs or other intellectual property rights of any third party; or

10.2.2 an obligation on RI or EPA to bring or prosecute or defend actions or suits against or by third parties for infringement of patents, copyrights, trademarks, industrial designs or other intellectual property or contractual rights, whether in connection with its Pre-Collaboration IP or the Project IP developed under this Agreement or otherwise.

10.3 No action whether in contract or tort (including negligence) or otherwise arising out of or in connection with this Agreement may be brought by a Party against the other more than [redacted] years after the course of action has accrued.

10.4 Save for death or personal injuries caused by negligence, in no event shall an A*STAR RI, whether as a breach of contract, tort or otherwise, have any liability to EPA or to a third party for any indirect, special, incidental, consequential damages, loss of profits or pure economic loss.

10.5 EPA's responsibility for the payment of claims to the A*STAR RI or its employees for personal injury or death caused by the negligence or the wrongful act or omission of employees of EPA, while acting within the scope of their employment, shall be in accordance with applicable laws.

10.6 Notwithstanding anything to the contrary, the A*STAR RIs' total and cumulative liability under this Agreement, however arising, shall not exceed Singapore Dollars [redacted].

11. USE OF NAMES

No Party shall issue any press release relating to this Agreement without obtaining the prior written consent of the other Parties. Prior to being released or made, a copy of all press releases which a Party intends to issue or make regarding this Agreement shall be provided to the other Parties for approval, which approval shall not be unreasonably withheld.

12. TERMINATION

12.1 Any of the Parties shall be entitled to terminate this Agreement immediately by notice in writing to the other Parties (but without prejudice to any rights any Party may have against the other arising prior to such termination) if any of the events set out below shall occur. The said events are:

12.1.1 if a Party shall commit any material breach of any of its obligations under this Agreement and shall fail to remedy such breach (if capable of remedy) within [redacted] days after being given notice by the first Party so to do; or

12.1.2 if a Party (being a company) shall go into liquidation whether compulsory or voluntary (except for the purposes of a bona fide reconstruction or amalgamation with the consent of the first Party, such consent not to be unreasonably withheld) or if a Party shall have an administrator appointed or if a receiver, administrative receiver or manager shall be appointed over any part of the assets or undertaking of that other Party.

12.2 Pursuant to Clause 3.2, the A*STAR RIs shall be entitled to terminate this Agreement if the events specified in Clause 3.2 hereof occur, in which case the A*STAR RIs shall be relieved of its obligations herein (except for the obligations described in Clause 13.2 and any other obligations that are expressed to survive termination of this Agreement) and shall have no liability whatsoever to EPA in respect of such termination.

13. CONSEQUENCE OF TERMINATION

13.1 The provisions of Clauses 6, 7, 8, 9, 10, 11, 12.2, 13, 14, 18, 19, 20 and 21 shall continue in full force and in accordance with their terms, notwithstanding the expiration or termination of this Agreement for any reason.

13.2 Without prejudice to any claims for damages that either Party may be entitled to, upon termination or expiration of this Agreement, each Party shall promptly return all materials of the other Parties in its possession, including, without limitation, Confidential Information of the other Parties, upon the request of the other Parties.

14. ASSIGNMENT

14.1 Save as expressly provided in this Agreement, no Party shall assign this Agreement or otherwise transfer its rights or obligations, or any part thereof, under this Agreement without the prior written consent of the other Parties.

14.2 It is agreed that if at any time after the date of this Agreement the functions and operations of an A*STAR RI are assigned, merged, transferred into or otherwise forms part of another organization of A*STAR ("the New Entity"), such that the New Entity takes over the whole or substantially the whole of that A*STAR RI's operations, then it is agreed that that A*STAR RI may:

14.2.1 at its option, assign this Agreement in its entirety to the New Entity which will then assume all of RI's rights and obligations hereunder; or

14.2.2 assign all or any part of its rights hereunder to the New Entity.

15. FORCE MAJEURE

15.1 No Party shall be liable for delays in delivery or performance when caused by any of the following which are beyond the actual control of the delayed Party: (i) acts of God, (ii) acts of the public enemy, (iii) acts or failure to act by the other Party, (iv) acts of civil or military authority, (v) governmental priorities, (vi) hurricanes, (vii) earthquakes, (viii) fires, (ix) floods, (x) epidemics or pandemics, (xi) embargoes, (xii) war, and (xiii) riots (hereinafter referred to as the "Force Majeure Event").

15.2 The respective obligations of the Parties hereunder shall be suspended during the time and to the extent that such Party is prevented from complying therewith by a Force Majeure Event provided that such Party shall have given written notice thereof, specifying the nature and details of such event and the probable extent of the delay to the other Parties.

15.3 In case of a Force Majeure Event, the time for performance required by each Party under this Agreement shall be extended for any period during which the performance is prevented by the event. However, the other Parties may terminate this Agreement by notice if such an event prevents performance continuously for more than [redacted] days.

16. DISPUTE RESOLUTION

[redacted]

17. NOT IN USE

18. NOTICE

18.1 Any notice to be given by any Party to this Agreement shall be in writing and shall be deemed duly served if delivered personally or sent by facsimile transmission or by prepaid registered post to the addressee at the address as stated above or (as the case may be) the facsimile number of that Party or at such other address (or facsimile number) as the Party to be served may have notified the other Parties for the purposes of this Agreement.

18.2 Any notice sent by facsimile shall be deemed served when despatched and any notice served by prepaid registered post shall be deemed served forty-eight (48) hours after despatch thereof. In proving the service of any notice it will be sufficient to prove in the case of a letter that such letter was properly stamped addressed and place in the post or delivered or left at the current address if delivered personally and in the case of a facsimile transmission was duly despatched to the facsimile number of the addressee given above or subsequently notified for the purposes of this Agreement.

19. EXPORT CONTROL

EPA shall ensure that it and its end-users of any IP licensed or otherwise made available to EPA shall comply with all applicable laws, rules and regulations governing the use, export and disposal of the IP, including those related to export control.

20. ENTIRE AGREEMENT

Unless otherwise expressly specified, this Agreement embodies the entire understanding between the Parties in respect of the Project and any prior or contemporaneous representations, either oral or written, are hereby superseded. No amendments or changes to this Agreement shall be effective unless made in writing and signed by authorized representatives of the Parties.

21. GENERAL

21.1 No exercise or failure to exercise or delay in exercising any right power or remedy vested in any Party under or pursuant to this Agreement shall constitute a waiver by that Party of that or any other right power or remedy.

21.2 The Parties shall co-operate with each other and execute and deliver to the other Party such instruments and documents and take such other action as may be reasonably requested from time to time in order to carry out and confirm the rights and the intended purpose of this Agreement.

21.3 In the event that any term, condition or provision of this Agreement is held to be a violation of any applicable law, statute or regulation the same shall be deemed to be deleted from this Agreement and shall be of no force and effect and this Agreement shall remain in full force and effect as if such term condition or provision had not originally been contained in this Agreement. Notwithstanding the above in the event of any such deletion the Parties shall negotiate in good faith in order to agree the terms of a mutually acceptable and satisfactory alternative provision in place of the provision so deleted.

21.4 This Agreement may be executed in any number of counterparts or duplicates each of which shall be an original but such counterparts or duplicates shall together constitute but one and the same agreement.

21.5 The recitals and schedules of this Agreement shall form an integral part of this Agreement.

21.6 It is agreed that for the purposes of this Agreement, "IBN" shall mean the Institute of Bioengineering and Nanotechnology only, and "BII" shall refer to the Bioinformatics Institute only. Reference to IBN and BII herein shall not extend to any other research institute, center or division of the Biomedical Sciences Institutes. For avoidance of doubt, no research institute, center or division within the Biomedical Sciences Institutes (other than BII and/or IBN, as the case may be) shall have any obligation under this Agreement to the EPA or to disclose to or receive from the EPA any information unless expressly agreed in writing.

22. THIRD PARTY CONTRACTS ACT

Save for the parties identified in Clauses 7, 8 and 14, the Parties do not intend that any term of this Agreement should be enforceable by any person or entity who is not a party to this Agreement.

AS WITNESS the hands of the Parties hereto the day and year first above written.

SIGNED by)
)
for and on behalf of)
)
Institute of Bioengineering and)
Nanotechnology)
)
in the presence of:)

_____
[Name/title of signatory]

_____
[Name/title of Witness]

SIGNED by)
)
for and on behalf of)
)
Bioinformatics Institute)
)
in the presence of:)

_____
[Name/title of signatory]

_____
[Name/title of Witness]

SIGNED by)
)
for and on behalf of)
)
United States Environmental Protection)
)
Agency, in the presence of:)

_____
[Name/title of signatory]

_____
[Name/title of Witness]

SCHEDULE 1

PROJECT PLAN

Human *in vitro* models for the prediction of nephrotoxicity of environmental and industrial chemicals

1. SCOPE OF WORK AND BACKGROUND

Scope of the work is the establishment and characterization of *in vitro* models for the prediction of the renal proximal tubular (PT) toxicity of environmental and industrial chemicals in humans. Furthermore, the *in vitro* models should be applied for predicting the human PT toxicity of [redacted] ToxCast compounds with unknown nephrotoxicity in humans. A longer term goal is the regulatory acceptance of the *in vitro* models and a respective proposal to FDA should be submitted during the current funding period.

The kidney is a major target organ for chemical-induced toxicity, both drugs and environmental chemicals, and injury to the renal PT is frequently observed (Choudhury and Ahmed, 2006; Gil et al., 2005; Jarup and Akesson, 2009; Naughton, 2008; Tiong et al., 2014; Vaziri et al., 1979). This is due to the roles of the PT in clearing compounds from the bloodstream by active transport into the glomerular filtrate, in reabsorbing compounds from the glomerular filtrate and in compound metabolism (Lash et al., 2006; Lash et al., 2008; Lohr et al., 1998; Morrissey et al., 2013; Morrissey et al., 2012). The ToxCast models have not covered nephrotoxicity yet due to a lack of *in vitro* models that can reliably predict nephrotoxicity, and in particular PT toxicity (a comprehensive overview over current renal *in vitro* models and their predictivity is provided in (Tiong et al., 2014)).

Recently, the group of D. Zink (IBN) has established the first *in vitro* models for the prediction of renal PT toxicity in humans. These models employed human primary renal proximal tubular cells (HPTC) (Li et al., 2013) or pluripotent stem cell-derived HPTC-like cells (Li et al., 2014). Pre-validation was performed with a set of 41 compounds, which comprised drugs as well as environmental and industrial chemicals (Li et al., 2014; Li et al., 2013). All compounds had well-characterized effects on human kidneys and PT (listed in detail in (Li et al., 2014)). Endpoints of the *in vitro* models were expression of interleukin (IL)6 and IL8 determined by qPCR. It was demonstrated that use of these endpoints resulted in much higher predictivity than use of other commonly applied endpoints (Li et al., 2014). Computational analysis of the results by using machine learning methods further improved the predictivity (Su et al., 2014). The computational work was performed by the group of Lit Hsin Loo (BII).

More recently, an *in vitro* model based on human induced pluripotent stem cell (iPS)-derived HPTC-like cells has been developed, which also employed machine learning methods (Kandasamy, Chuah et al., in press). This predictive model had a training balanced accuracy of 99% and a test balanced accuracy of 87%, and also accurately identified injury mechanisms. Whereas this model still used IL6/IL8 expression as endpoint, the groups of D. Zink and L. H. Loo are currently developing novel renal models that combine high content screening (HCS) with phenotypic profiling. Such human renal *in vitro* models combine high predictivity with high efficiency and are suitable for the assessment of large numbers of compounds. Phenotypic profiling platforms have been developed in L. H. Loo's group (Laksameethanasan et al., 2013; Loo et al., 2007). The current models based on HCS combined with phenotypic profiling have training balanced accuracies of >99% and test balanced accuracies of ~76% to 89% with respect to predicting PT injury in humans, depending on the cell type used. One advantage of phenotypic profiling is

that high predictivity can be obtained without any knowledge of the injury mechanisms. However, the phenotypic changes often indicate the injury mechanisms involved, which can be further addressed if required.

This technology will be further explored in the collaborative project with K. Houck (EPA) in order to establish *in vitro* models for the prediction of PT toxicity of environmental and industrial chemicals in humans. The objectives are the following:

Objectives

1. **Screening of ~ 40 - 50 compounds with known PT toxicity in humans.** For establishing the *in vitro* models and for determining their predictivity with respect to human PT toxicity ~ 40 – 50 environmental toxicants and industrial chemicals will be screened. All selected compounds should have well-characterized effects on human PTs and kidneys (toxic or not toxic). As many ToxCast compounds as possible should be included, depending on the availability of results on their nephrotoxicity in humans. For all selected compounds also results on the nephrotoxicity in other species should be available. [redacted]

2. **Screening for the most discriminative phenotypic features and markers for binary nephrotoxicity prediction.** Large numbers [redacted] of phenotypic features will be automatically quantified from the HCS images obtained in Objective 1, and used to construct high-dimensional dose response curves. The prediction performances of different features and properties of their dose response curves, such as [redacted], will be systematically evaluated. [redacted]

3. **Establishment of *in vitro* models for predicting the PT toxicity of environmental and industrial chemicals in humans.** Result of the work performed under Objectives 1 and 2 would be pre-validated *in vitro* cell and computational models with well-characterized predictivity with respect to human PT toxicity. The distinguishing feature of the different models would be the cell types used [redacted], and for each model and cell type used the predictivity would be known. The *in vitro* models would be binary and would provide a yes/no answer with respect to PT toxicity in humans, but would not provide information with respect to the *in vivo* dose response.

4. **Predicting the *in vivo* dose response from *in vitro* results.** The work under Objective 2 would generate *in vitro* dose response results for all of the ~ 40 - 50 compounds screened. In addition, for all of the compounds human and/or and other species dose response results should be obtained from ToxRefDB, RTECS or other sources. Together, these results will be used for the development of computational models that predict the *in vivo* dose response from *in vitro* results with respect to human PT toxicity. [redacted]

5. **Confirmation of the correct indication of injury mechanisms by the *in vitro* models.** About 10 compounds will be selected from the set of ~ 40 - 50 compounds in order to address whether injury mechanisms that are relevant *in vivo* are correctly triggered in the *in vitro* models. For this work compounds where the injury mechanisms have been well characterized *in vivo* (preferably in humans) will be selected. Injury mechanisms [redacted] will be addressed *in vitro* by selecting suitable endpoints and assays.

6. **Proposal on regulatory acceptance and work guided by FDA.** One of the

main goals is regulatory acceptance of the human PT-specific predictive *in vitro* models, especially of the models that combine HCS with phenotypic profiling. Discussions with FDA will be facilitated by EPA though the Tox21 Memorandum of Understanding (http://www2.epa.gov/sites/production/files/2014-08/documents/mou_epa-ntp-ncgc-fda.pdf). A proposal on regulatory acceptance will be submitted to FDA after completion of work on Objectives 1 – 5. Further work required for regulatory acceptance will be performed as advised by FDA and under the guidance of FDA.

7. **Predicting the human PT-toxicity of a larger set of ToxCast compounds with currently unknown effects on human kidneys.** [redacted] ToxCast chemicals with unknown effects on human kidneys will be screened. The exact number of compounds may be changed to reflect the required stock concentrations determined by Objective 1. The screening work will be performed in similar ways as described under Objective 1 above and the *in vitro* model with the best performance (predictivity and injury mechanisms) will be selected for the work. PT toxicity of the [redacted] compounds as well as the dose response in humans will be predicted based on the screening results and the results of the work performed under Objectives 1 - 4. The opportunity to predict the PT toxicity of compounds with currently unknown effects on human kidneys would be of great translational value.

[redacted]

3. DELIVERABLES

1. Pre-validated *in vitro* model [redacted] with determined predictivity for the prediction of the PT toxicity in humans of industrial and environmental chemicals.
2. Pre-validated *in vitro* model [redacted] with determined predictivity for the prediction of the PT toxicity in humans of industrial and environmental chemicals.
3. Pre-validated *in vitro* model [redacted] with determined predictivity for the prediction of the PT toxicity in humans of industrial and environmental chemicals.
4. Confirmed correct identification of injury mechanisms with respect to the three models listed under points 1-3.
5. Documentation for the computational procedures used to generate the predictivity values for the purpose of understanding and interpreting the values. This documentation will be provided in the form of text and/or flow charts in a PDF/Word file.
6. Prediction of the PT toxicity of [redacted] ToxCast chemicals in humans.
7. Prediction of the *in vivo* dose response of [redacted] ToxCast chemicals with respect to PT toxicity in humans.
8. Proposal for regulatory acceptance to FDA.
9. Two annual reports (Year 1 and Year 2).
10. Final report.

4. INPUTS TO THE PROJECT / RESOURCES

4.1. IBN Existing Resources

D. Zink's lab at IBN is fully equipped for the planned work. The existing resources include common lab equipment (fridges, freezers, centrifuges etc.), an HCS machine with attached computers and servers for image analysis and data

storage, an automated liquid dispenser and plate washer, qPCR machines, a microplate reader and an epifluorescence microscope. Cell culture facilities are available, which include biosafety cabinets, incubators, microscopes, centrifuges, a cell counter and liquid nitrogen tanks.

4.2. IBN IP

D. Zink's lab has many years of experience in handling and characterizing renal cell lines and HPTC. D. Zink's lab was involved in developing the first protocol for the differentiation of human pluripotent stem cells into HPTC-like cells (Narayanan et al., 2013) and has developed the currently most rapid and efficient protocol for generating iPSC-derived HPTC-like cells (Kandasamy, Chuah et al., in press). Furthermore, D. Zink's lab has developed the first *in vitro* models for the prediction of PT toxicity in humans (Li et al., 2014; Li et al., 2013). Predictive renal models that combine HCS and phenotypic profiling are currently established in close collaboration with L. H. Loo's lab.

4.4 BII IP

BII has experience in developing experimental and computational methods for high-content screening and drug-response characterization. These methods include isolation and handling of primary human tumor cells, image-based drug-response assays, automated image acquisition and processing, and computational algorithms and software tools for quantifying cellular phenotypes and deriving drug profiles. BII has been using these methods to study and predict drug sensitivities, on- and off-target effects, and mechanisms.

5. PRE-COLLABORATION INTELLECTUAL PROPERTY

5.1 IBN IP

- Patent No. US 8,481,316 B2, "Method for differentiating embryonic stem cell into cells expressing AQP-1", A*STAR
- PCT Application No. PCT/IB2013/001589, "In vitro assay for predicting renal proximal tubular cell toxicity" A*STAR
- PCT Application No. PCT/IB2013/001944, "In vitro assay for predicting renal proximal tubular cell toxicity" A*STAR
- PCT Application No. PCT/SG2014/000529, "Method for differentiating induced pluripotent stem cells into renal proximal tubular cell-like cells", A*STAR
- PCT Application No. PCT/SG2015/050039 "Method for predicting toxicity of a compound based on nuclear factor-κB translocation", A*STAR

5.2 BII IP

- cellXpress, Cellular Phenotypic Profiling and Analysis Software Platform. Copyrighted 2011-2015, Bioinformatics Institute, A*STAR, Singapore.
- [redacted]

5.3 EPA IP

- TOXCAST database

6. FUNDING and MANAGEMENT SUPPORT

- [redacted]
- Management of the A*STAR part of the project will be supported by IBN's and BII's administration

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