COOPERATION AGREEMENT

This Cooperation Agreement (the "**Agreement**") for the purpose of **"TM05000031 – Targeting the Androgen Receptor: Developing Binder Molecules for Prostate Cancer Therapy and Advancing NMR-AI Platform for Enhanced Drug Design"** is made as of 13 July, 2023 (the "**Agreement Date**"), by and between the Contractual Parties including Project Leader, Project Partner and Project Participants **(**collectively as **"All Contractual Parties"** and each, an **"Contractual Party"):**

Project Leader
Name: **AI|ffinity s.r.o.**
Id. No.:
Registered seat: JIC INMEC, Purkyňova 127, 612 00 Brno-Medlánky, Czech Republic
Represented by: Thomas Evangelidis, M.Res., M.Phil., Ph.D., CEO & CSO
(the "**Receiver**"); and,

Project Partner
Name: **AnHorn Medicines Co., Ltd.**
Id. No.: 83523270
Registered seat: 5F, No. 99, Ln. 130, Sec. 1, Academia Rd., Nangang Dist., Taipei 115, Taiwan (R.O.C.)
Represented by: Chu-Chiang Lin, Founder & CEO
(the "**Partner**"); and,

Project Participants
Name: **NexMR GmbH**
Id. No.: CHE-461.095.635
Registered seat: IFJ Institut für Jungunternehmen AG, Wiesenstrasse 10A, 8952 Schlieren, Switzerland
(the "**NexMR**"); and,

Name: **Masaryk University, Central European Institute of Technology**
Id. No.: CZ00216224
Registered seat: Žerotínovo nám. 9, 601 77 Brno, Czech Republic
(the "**CEITEC MU")**

(the "**Participants**")

Preamble

All Contractual Parties cooperate in the implementation of Project No. **TM05000031** entitled "**TM05000031 - Targeting the Androgen Receptor: Developing Binder Molecules for Prostate Cancer Therapy and Advancing NMR-AI Platform for Enhanced Drug Design**" (the "**Project**"), which the Receiver submitted to the 5th public tender of the DELTA 2 support programme for applied research, experimental development, and innovation (the "**Programme**") of the Technology Agency of the Czech Republic (the "**Provider**").

Provided that the Provider enters into the Agreement on Granting of Subsidy to the Project with the Receiver (the "**Agreement on Granting of Subsidy**"), All Contractual Parties undertake herein to cooperate in Project implementation and in utilizing the results of the Project.

Article I

Subject Matter of Agreement

1.1 The subject matter of this Agreement is to describe the roles and rights and obligations of All Contractual Parties relating to the implementation of the Project, in particular to define the rights and duties of All Contractual Parties with respect to (i) rights to intangible property (e.g. intellectual property) necessary for the implementation of the Project, (ii) rights to intangible property created during or in relation to the Project and (iii) regulation of utilizing the results of the Project.

1.2 The nature, purpose, goals and expected results of the Project are specified (i) in the Project proposal registered with the information/application system of the Provider and (ii) in the responsible assignments which form **Annex 1** hereof.

Article II

Terms and Conditions of Cooperation apply to All Contractual Parties

2.1 All Contractual Parties shall cooperate in compliance with the proposed Project and other conditions and documents that are binding for the Project. All Contractual Parties become acquainted with the Project content before signing this Agreement, including the Project application and all Programme conditions.

2.2 All Contractual Parties undertake to use all necessary efforts in order to achieve the purpose, goals and expected results of the Project as defined in Annex 1 to this Agreement. Failure to accomplish the purpose, goals and/or expected results of the Project may only be justified by circumstances generally recognized and defined as *force majeure*.

2.3 All Contractual Parties undertake to act and perform in a manner that will not jeopardize the implementation of the Project and the interests of the Participants and the Investigators (as defined in Article III below).

Article III

Structure of the Project – Participants and the Investigators (collectively as "All Investigators")

3.1 The person responsible for the scientific implementation of the Project by the **Receiver** is the principal investigator: xxxxxxxxxxx, email: xxxxxxxxxxxxx
telephone: +xxxxxxxxxxxx, address: JIxxxxxxxxxx

3.2 The person responsible for the scientific implementation of the Project by the **Partner** is the responsible investigator: xxxxxxxxxxxxxxxxxxxxx

3.3 The person responsible for the scientific implementation of the Project by **NexMR** is the responsible investigator: xxxxxxxxxxxxxxxxx

3.4 The person responsible for the scientific implementation of the Project by the **CEITEC MU** is

the responsible investigator: xxxxxxxxxxxxxxxxxxxxxxxxxx
3.5 The investigators are involved in the activities necessary for the successful completion of the Project in compliance with the approved Project proposal.

Article IV
Project Management, Involvement of Respective Contractual Party in Project
4.1 The Receiver is the Project submitter and applicant for the provision of subsidy in the Czech Republic. The Receiver shall conclude an Agreement on Granting of Subsidy with the Provider. The Receiver is the coordinator of the Project and provides administrative cooperation with the Provider in the Czech Republic.
4.2 The Partner is an applicant for the provision of subsidy in the country of its origin under the terms and conditions applicable in the country where the subsidy is granted.
4.3 The Partner undertakes to exercise all necessary efforts to implement the Project, and to act in a manner that will not jeopardize the implementation of the Project, the Project goals and results and the interests of the Receiver and the Participants. The Receiver undertakes to exercise all necessary efforts to implement the Project, and to act in a manner that will not jeopardize the implementation of the Project, the Project goals and results and the interests of the Partner and the Participants.
4.4 All Contractual Parties and All Investigators undertake to perform within the set deadlines and defined extent the activities leading to the Project implementation as specifically determined in the Project proposal and/or any other activities as necessary or needed for proper Project implementation.

Article V
Course and Evaluation of Project
5.1 For the purposes of verification and evaluation of progress in the Partner's cooperation during the Project implementation, All Contractual Parties are obligated to provide any and all relevant progress information and documents during the monthly meeting; and each party is responsible to prepare reports for their country.
5.2 All Contractual Parties undertake to cooperate on the execution of implementation plan to the Project results.

Article VI
Rights and Duties to All Contractual Parties
6.1 All Contractual Parties are obligated to notify each other any and all changes concerning the Project, any inability to perform obligations under this Agreement duly and in a timely manner and any and all material changes and facts that could affect the implementation, expected results and goals of the Project no later than seven (7) calendar days from the day on which they become aware thereof. All Contractual Parties are further obligated to prove at any time that they remain qualified to participate in the Project implementation.
6.2 All Contractual Parties undertake to archive documents relating to the Project for at least ten (10) years from the completion of the Project.

Article VII
Intellectual Property, Tangible Property
7.1 This Agreement governs the rights and obligations of All Contractual Parties to the existing intellectual property prior to entering into this Agreement (the "**Pre-Existing Knowledge**") and sets forth the rules of utilization of such Pre-Existing Knowledge for the purposes of implementation of the Project. Further, the Agreement governs the rights and obligations of All Contractual Parties to intellectual property created during the term hereof.

7.2 Intellectual property for the purposes of this Agreement means any results of intellectual activity, based on which any objectively perceivable intangible property is created. In particular, this includes inventions, technical solutions protected as a utility model, industrial designs, innovations and rationalization proposals, biotechnological inventions, trademarks, copyrighted works, know-how and other results of an intellectual activity.
7.3 Pre-Existing Knowledge which is necessary for the implementation of the Project or the utilization of its results shall remain the property of respective Contractual Party, however such Contractual Party shall permit the other Contractual Party to use any of its Pre-Existing Knowledge to the extent as necessary for the purposes of implementation of the Project.
7.4 All Contractual Parties acknowledged a separate license agreement between the AI|ffinity and CEITEC MU, file n. 005-2022-Bur as amended on June 15, 2023, including right to option and back-license to CEITEC MU for the use of derivative works (the "**Separate License Agreement**"), shall not affect the Partner's freedom to use the Intellectual Property and Tangible Property created during the term of this agreement, and such usage shall be granted free of charge.
7.5 In the event of any inconsistency between this Agreement and the Separate License Agreement or any license agreements between each Contractual Party that were not fully disclosed to the Partner before the effective Agreement Date, this Agreement shall prevail.
7.6 All Contractual Parties agreed that the intellectual property rights of the Partner's AIMCADD platform and its technology, all the data generated by this platform, and all resulting hits, lead compounds, and related chemical structures generated from the Project are exclusively owned by the Partner. Furthermore, the Partner holds complete ownership over all experimental data generated during their research activities.
7.7 All Contractual Parties agreed that the intellectual property rights of AI|ffinity's upgraded NMR-AI software platform, all in silico data generated by this platform are the exclusive property of AI|ffinity. This includes all outsourced DNA vectors derived from the AR-V7 sequence, protein expression, and purification protocols and HDX-MS data. Moreover the processed NMR spectra (including chemical shift assignments) and all related structural data, processed X-ray and SAXS structural data generated at CEITEC CF, the 3D structures and MD trajectories of protein and protein-ligand complexes created by AI|ffinity in this project are unequivocally owned by AI|ffinity. This data can be utilized for the purpose of improving AI|ffinity's technology, but not for development and commercialization related to any Androgen Receptor applications unless the Receiver receives written approval from the Partner. AI|ffinity's use of 4D-GRAPHS software and other appropriate results remains subject to the Separate License Agreement. AI|ffinity's upgraded 4D-GRAPHS software and any derivatives will be available to CEITEC MU free of charge for noncommercial applications subject to the license agreement above.
7.8 All Contractual Parties agreed that NexMR is the sole owner of the intellectual property rights associated with its photo-CIDNP spectra collection, photo-CIDNP compatible fragment library, experimental methodologies, hit discovery and characterization methods, as well as any methodological development by a NexMR employee. In addition, NexMR shall have the sole ownership and rights to use all experimental data, including binding data, affinity data, and epitope restraints, derived from photo-CIDNP NMR experiments in this Project. This data can be utilized for the purpose of improving NexMR's technology and may be disclosed for this purpose, but not for commercialization.
7.9 All Contractual Parties agreed that the intellectual property rights of all raw data produced by measurements conducted at CEITEC CF, are solely owned by CEITEC MU. This data includes, but is not limited to, unprocessed NMR spectra, MST data, X-ray crystallography data, SAXS data, circular dichroism data, analytical size-exclusion chromatography (SEC) data, and any other biophysical measurements made at CEITEC CF. However, the ownership over all data and research outcomes generated from the processing of these raw data by AI|ffinity (characterization of protein-ligand interaction, Kd values, molecular shapes by SAXS, molecular weights by SEC, 3D structures), will belong to AI|ffinity, but not for development and commercialization related to any

Androgen Receptor applications unless the Receiver receives written approval from the Partner. CEITEC MU, receives compensation from the collaborating undertaking equivalent to market prices for the out-sourced services to the results assigned by the Project Leader of the Project. The arising activities and assigned to or accessed by the Project Leader undertaking after the conclusion of Project, predominantly the raw data produced shall be governed by the Separate License Agreement and without effect to the right of the Partner.

7.10 All Contractual Parties agreed that the Partner has unrestricted freedom to obtain and apply for internal R&D purposes all types of NMR and X-ray structural data of protein, protein-ligand, and related structural data derived from the Project.

7.11 All Contractual Parties agreed that the Partner has unrestricted freedom to submit patent applications in protection of the related chemical structures derived from the Project into the disease indications, including but not limited to, prostate cancer, breast cancer, liver cancer, lung cancer, bladder cancer, kidney cancer.

7.12 All Contractual Parties agreed that AI|ffinity and NexMR won't be listed as co-inventors and/or applicants on the patents for the therapeutic molecules. However, they will be the lead authors on any non-commercial scientific papers that get published. All Contractual Parties agree to provide appropriate acknowledgment of CEITEC MU as the source of the relevant data or relevant intellectual property in relevant publications and acknowledge CEITEC MU contributors as authors where appropriate.

7.13 All Contractual Parties agreed that the Intellectual property for which the protection is possible (patents, utility models etc.) cannot be disclosed until the respective applications for protection are submitted.

7.14 All Contractual Parties agreed to collaboratively choose suitable research findings, to be submitted for joint publication in a journal, after the patent application is submitted.

Article VIII
Ensuring Protection of Information and Outcomes Obtained in Connection with the Project

8.1 All Contractual Parties undertake to provide each other with all information as necessary to carry out the activities hereunder. Unless All Contractual Parties agree otherwise any and all information obtained from the other Contractual Party, which is not in the public domain is considered to be confidential (the "**Confidential Information**").

8.2 Any of the respective Contractual Party that has obtained such Confidential Information is obligated to maintain confidentiality thereof and ensure sufficient protection against unauthorized access thereto. It must not disclose such Confidential Information to any other person/entity, save for its employees and other persons who are in charge of conducting activities under this Agreement and with whom the respective Contractual Party has concluded a confidentiality agreement with a scope similar to that stipulated for All Contractual Parties by this Agreement, and it shall not use the Confidential Information for any purpose other than the performance of activities under this Agreement.

8.3 Duties pursuant to para 8.1 apply without any change and remain valid for a period of ten (10) years after the termination of this Agreement, notwithstanding the reason for such termination.

Article IX
Liability for Damage

9.1 All Contractual Parties acknowledge that a breach of a duty under this Agreement by any of the Contractual Party may result in the other Contractual Party incurring damage, and undertake to compensate the other Contractual Party for any damage so caused.

Article X

Final Provisions

10.1 This Agreement becomes valid on the date of its signature by All Contractual Parties and the rights and obligations to the **Article VII Intellectual Property, Tangible Property** shall be effective as of the signature date of the Agreement on granting of Subsidy. The Agreement is concluded for the duration of the Project and for three (3) years after the completion of the Project. All Contractual Parties have agreed that those provisions of the Agreement which were apparently intended by All Contractual Parties to survive after the termination or expiry of the Agreement shall remain valid and effective (in particular Articles 7.1 - 7.9 and Articles 8.1 - 8.3).

10.2 All Contractual Parties have agreed to settle any disputes arising out of the implementation of the Agreement by mutual agreement. Should such amicable settlement prove to be impossible within a reasonable amount of time, Any controversy or claim arising out of or relating to this Agreement or the breach thereof, shall be settled by arbitration administered by the Singapore International Arbitration Centre ("**SIAC**") in accordance with the Arbitration Rules of the Singapore International Arbitration Centre for the time being in force, which rules are deemed to be incorporated by reference in this Section. The seat of the arbitration shall be Singapore. The Tribunal shall consist of one (1) arbitrator to be appointed by the President for the time being of the SIAC. The language of the arbitration shall be English. The language to be used in the proceedings shall be in English.

10.3 The Agreement may cease to exist upon full discharge of all obligations by All Contractual Parties arising hereunder, and/or by a written agreement of All Contractual Parties in which the Receiver and the Partner agree upon the terms and conditions of the termination of the Agreement.

10.4 This Agreement shall be governed by and construed and enforced in accordance with the laws of Singapore. The terms and conditions of subsidy granted to each Contractual Party by its country of origin shall be governed by valid laws and regulations of the country granting such subsidy.

10.5 Changes and amendments to the Agreement may be made solely by agreement of All Contractual Parties in the form of written numbered amendments to the Agreement. The Partner is not entitled to transfer rights and duties hereunder to a third party without the prior written agreement of the Receiver during the Agreement on Granting of Subsidy.

10.6 This Agreement may be terminated under the following circumstances: (i) Any Contractual Party may terminate this Agreement if, upon receipt of written notice from the non-breaching party, the breaching party fails to rectify its breach of this Agreement within a reasonable period of time specified in the notice; or (ii) either the Receiver or the Partner may terminate this Agreement if the application for Granting of Subsidy is rejected.

10.7 An electronic copy, telecopy or other reproduction of this Agreement may be executed by one or more parties hereto and delivered by such party by e-mail or any similar electronic transmission device pursuant to which the signature of or on behalf of such party can be seen. Such execution and delivery shall be considered valid, binding and effective for all purposes. At the request of any party hereto, all parties hereto agree to execute and deliver an original of this Agreement as well as any electronic copy, telecopy or other reproduction hereof.

10.8 All Contractual Parties hereby declare that they have read through the whole Agreement, agree with the context and further represent that this Agreement has been concluded in full compliance with their internal policies and that they are fully aware of the obligations they assume by concluding this Agreement.

(signature page follows)

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Annex 1 – Responsible Assignments and Key Milestones of the Project

Name	Task Group	Task & Assignment	Key Milestones	Description
Receptor design, synthesis & structure determination [weight: 15%]				
Receptor design, synthesis & structure determination Executing company : AI|ffinity & CEITEC MU (weight: **100** %)	Protein engineering	Creation of protein expression constructs of C-terminus AI|ffinity (subcontracting)	Clone **several sequences** from the C-term (up to 171 aa), including the DNA-binding domain.	Design of **5 DNA constructs with different boundaries** and different affinity tags using standard gene synthesis / molecular cloning methods. Expression tests in LB medium - determination of the optimal conditions for each protein variant in soluble form.
		Optimization of expression/purification protocols AI|ffinity (subcontracting)	Express in E. coli, establish a high-yield **expression/purification protocol**. Select the longest possible, hereafter referred as AR-V7-Cterm.	Production of the 3 best constructs (1L in LB medium). 1. **Purification** on the soluble part by affinity chromatography (according to the tag), tag removal by TEV cleavage, reverse affinity chromatography and gel filtration. 2. **Standard Quality Control:** SDS-PAGE, maximal solubility concentration, storage conditions. 3. **Technical report** with SDS Page gels and chromatograms.
	Structure determination of the apo form	Apo structure shape determination by SAXS AI|ffinity & CEITEC MU	Determine the **shape** of the AR-V7-Cterm apo form.	**1. Data collection:** protein sample is exposed to an X-ray beam, and scattering patterns are recorded over a range of scattering angles. The intensity of scattered X-rays is measured as a function of the scattering angle. **2. Data processing:** background subtraction and normalization. **3. Data analysis and interpretation.** **Result:** general shape of AR-V7-Cterm apo form.
		Apo secondary structure analysis by Circular Dichroism AI|ffinity & CEITEC MU	Estimate the **secondary structure (SS) element content** (alpha-helices, beta-sheets, and random coils) in the AR-V7-Cterm apo form.	The primary data collected in a CD experiment is the differential absorbance or CD signal, typically represented as the molar ellipticity (θ). Experimental steps: **1. Data Collection:** Collect the

				CD spectrum of protein sample by scanning a range of wavelengths (typically in the UV or visible range) while measuring the differential absorbance. **2. Data Analysis:** Analyze the collected CD spectra - apply mathematical algorithms and/ or compare the experimental data to reference datasets or known spectral features. **3. Interpretation** **Outcome:** qualitative and quantitative information about the secondary structure content and folding behavior of the AR-V7-Cterm apo form.
		Production of isotopically labeled protein for all NMR experiments AI|ffinity	Feasibility study to assess the potential success and efficiency of the labeling process, ensuring the optimum use of resources and accurate results. Subsequently **productions of AR-V7-Cterm protein labeled with 15N, 13C or 2D**, depending on the NMR experiment.	All the NMR tasks throughout the project will rely on labeled samples of AR-V7-Ctem. As the expression of labeled proteins may significantly decrease the yield, a feasibility study of such production will have to take place before production. If production is attainable, two highly pure and concentrated samples of the AR-V7-Ctem (~0.3 mM), one labeled with ^15N, ^13C and ^2D and the other with just ^15N and ^13C for NMR signal enhancement, will be produced for 4D NMR structure determination. Simultaneously a larger quantity of ^15N labeled sampled will be produced for all 2D NMR experiments for compound binding validation, mapping of interactions with the protein and estimation of Kd.
		Structure determination by 4D-NMR or X-ray AI|ffinity & CEITEC MU	Determine the **structure** of the AR-V7-Cterm apo form.	The primary data collected in **4D-NMR experiments** are NMR spectra, containing information on resonance frequencies, intensities, and connectivity patterns of the nuclei in the biomolecule. The analysis of these spectra can provide

				detailed structural information, including the coordinates of atoms, bond lengths, dihedral angles, and other structural parameters of the biomolecule. Moreover, the technique enables the determination of the spatial arrangement of atoms and the folding of the molecule in solution, providing insights into its function and interactions with other molecules. **Sample Preparation (outsourced):** check "Production of isotopically labeled protein for NMR experiments" task. **1. Data Collection:** Collect NMR spectra by measuring the resonance frequencies and intensities of the nuclei in the sample. From the first sample the NH(CO)CACB spectrum will be recorded, while from the second sample the 4D HCNH NOESY spectrum. The four dimensions in the last spectrum refer to the four frequency domains recorded, typically proton (1H), carbon (13C), amide nitrogen (15N), and amide hydrogen (1H) nuclei. **2. Data Analysis:** Fourier transformation, phasing, and baseline correction to obtain frequency and intensity information. Resonance assignment to peaks will be conducted automatically using AI|ffinity's 4D-GRAPHS algorithm. **3. Structure Calculation:** Use specialized software and algorithms to calculate the three-dimensional structure of the biomolecule from the assigned 4D NOESY spectrum. **Outcome:** 3D conformational ensemble of the AR-V7-Cterm apo form in solution. If 4D NMR structure determination fails, then we shall pursue X-ray crystallography. **Type of Data Collected:** The

				primary data collected in X-ray crystallography is the set of X-ray diffraction images, which contain the intensities and positions of the diffracted X-ray spots. **1. Sample Preparation:** crystallizing of the protein sample under controlled conditions **2. Data Collection:** expose the crystal to an intense and collimated X-ray beam. Rotate the crystal to different orientations and collect a series of X-ray diffraction images. **3. Data Analysis:** Process the collected X-ray diffraction images to obtain the intensities and positions of the diffracted X-ray spots. This involves indexing the diffraction pattern to determine the unit cell parameters and applying corrections for various factors such as background noise and detector distortions. **4. Structure Solution:** Use specialized software and algorithms to determine the phases of the diffracted X-ray waves. **Outcome:** detailed atomic-level information about the three-dimensional arrangement of atoms within the crystal lattice: positions, bond lengths, bond angles, and other structural parameters of the atoms in the material. From those, the average 3D structure of the AR-V7-Cterm in the crystal lattice will be derived.
Development of binder molecules for AR & AR-V7 [weight: 70%]				
2.1 Hit identification (weight: 30%) Executing company : AnHorn Medicines (weight: **50** %)	In silico-based screening	Preparation of AR-V7 DBD structure AnHorn Medicines	Establish **1** ligand binding pocket from the AR DBD structure that can be used for ligand screening	To predict the binding conformation of compounds to AR-V7 DBD, it is necessary to obtain the 3D structure of the protein. The researchers at AnHorn Medicines have downloaded the relevant structure of AR DBD from the RCSB Protein Data Bank

AI|ffinity & NexMR (weight: **50** %)				in the United States (https://www.rcsb.org/). The corresponding PDB code is 1R4I.
		Preparation of ligand database AnHorn Medicines	Prepare **1** compound database and complete the preprocessing	To predict the binding conformation of compounds to AR-V7 DBD, another essential requirement is to prepare a virtual drug molecule database. In this study, the researchers plan to use the ZINC15 virtual drug molecule database (https://zinc15.docking.org/) .
		Virtual screening AnHorn Medicines	Select **20-30** potential small molecules based on the screening results	AR DBD contains two zinc finger domains: the P-box, which spans from residue 577 to 581, and the D-box, which spans from residue 596 to 600. According to a published study by Huifang et al. in 2014, the main ligand binding pocket of AR DBD is located in the P-box. We have selected the P-box as the ligand binding pocket and will perform virtual screening using the AIMCADD Drug Development Platform.
	NMR-AI screening	1D NMR screening (Photo-CIDNP) NexMR	Screen **500** diverse fragments (in-house library) complemented by **50** (ordered) diverse compounds selected in collaboration with AI|ffinity.	Photo-CIDNP fragment screening is a novel method that utilizes nuclear magnetic resonance (NMR) for studying ligand-target interactions, overcoming traditional NMR limitations such as low sensitivity and long acquisition times. This method employs a technique known as photochemically induced dynamic nuclear polarization (photo-CIDNP), which hyperpolarizes the sample in an aqueous solution at room temperature by merely shining light on it. It facilitates the identification of hyperpolarizable small molecules, the creation of a

				library compatible with photo-CIDNP, and semi-quantitative assessment of ligand binding to a protein using photo-CIDNP quenching. This approach increases the throughput, reduces sample concentration, and allows for the detection of ligand-protein interactions using photo-CIDNP. Photo-CIDNP increases signal-to-noise ratio and enables the identification of binding through polarization quenching, even at low micromolar concentrations. The process leverages single-scan NMR experiments that last only 2 to 5 seconds and a high-throughput screening rate of 1500 samples per day, thanks to an automated flow-through platform. The availability of a photo-CIDNP fragment library further facilitates comprehensive fragment-based screening, accelerating ligand-target interaction screening in drug discovery. The photo-CIDNP NMR screening process involves several steps: **1. Identification of Hyperpolarizable Molecules:** This initial step focuses on finding small molecules that can be hyperpolarized using photo-CIDNP, i.e., their nuclear spin states can be altered upon exposure to light, leading to an enhancement of the NMR signal. **2. Creation of a Compatible Library:** The identified hyperpolarizable molecules are used to form a library that is compatible with the

				photo-CIDNP method. **3. Induction of Hyperpolarization:** The selected small-molecule library is exposed to light, inducing hyperpolarization and enhancing the sensitivity of the NMR measurement. **4. Assessment of Ligand-Protein Binding:** The hyperpolarized sample is then subjected to NMR, and the degree of photo-CIDNP quenching is assessed. This quenching is a result of ligand-protein interactions, allowing for the semi-quantitative evaluation of ligand binding to the target protein. **5. Screening of Fragments:** The fragments in the library are screened against the target protein, typically through high-throughput methods. The method has been applied to screen fragment molecules against proteins like PIN1, demonstrating its effectiveness and utility in detecting ligand-protein interactions.
		Adaptation of deepHitMiner to utilize 1D NMR photo-CIDNP data AI|ffinity & NexMR	**Modify deepHitMiner to leverage 1D NMR photo-CIDNP data** to improve its bioactivity predictions.	The deepHitMiner update will consist on the following steps: **1. Add new parsing functions** to read 1D NMR photo-CIDNP data of compounds interacting with **flexible proteins**. **2. Overhaul the core AI functions** to leverage the information of the new photo-CIDNP data. **3. Fix** any **bugs** or **limitations** detected during the field use. Once these steps are fulfilled, the new deepHitMiner version will be ready for application and

				licensing.
		Virtual screening guided by 1D NMR in incremental feedback loops (multiple rounds) AI|ffinity & NexMR	Train deepHitMiner, screen VS library (e.g., Enamine 220,000 Fragment Collection), select **~30** chemically diverse top-scored compounds for experimental validation.	The chemical library will be prepared with standard industry tools (LigPrep), compound geometries will be optimized and SQM partial charges will be computed using third-party software. A deepHitMiner model will be trained with specially designed feature vectors (2D molecular representations) that encompass structural information derived from the analysis of photo-CIDNP spectra. This model will be used to score the molecules in the chemical library and assign a probability in [0,1] (the higher the more probable the molecules to bind to the protein). The ~1000 top-scred compounds will be fed to our diversity selector algorithm to select ~30 of them that are structurally diverse, have good solubility, are synthetically accessible, contain interesting chemical groups (e.g. ligand epitopes from photo-CIDNP screening) and don't contain chemical groups that confer toxicity or promiscuity. Finally, selected top-scored analogs will be selected for synthesis by AnHorn or ordered from vendors. These ~30 compounds, which will be either ordered or synthesized by AnHorn, will be subjected to experimental validation of binding to the AR-V7-Cterm. This procedure will be repeated in incremental feedback loops, each time selecting new molecules for experimental validation,

				solving their 3D structure in complex with the receptor and retraining deepHitMiner. Optionally, once we obtain the first 3D structures of P-L complexes we will shift to structure-based drug design with 3D pharmacophore feature vectors encompassing structural information from photo-CIDNP data.
2.2 Hit validation (weight: 35%) Executing company : AnHorn Medicines (weight: **30** %) AI|ffinity & NexMR & CEITEC MU (weight: **70** %)	Binding validation and binding site characterization	Binding site mapping of hits by HDX-MS (optional) AI|ffinity	Run non-equilibrium, millisecond HDX-MS, which is suitable for IDPs, **identify binding residues** for each hit.	Binding site mapping by Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS) is a technique used to identify the regions of a protein that undergo changes in hydrogen-deuterium exchange upon binding to a ligand or interacting partner. **Type of Data Collected:** Mass spectra that provide information about the deuteration levels of the peptides. Experimental Steps: **1. Sample Preparation:** Prepare the protein-ligand complex. **2. Deuteration:** Exchange the labile hydrogen atoms in the protein with deuterium by incubating the protein in a deuterated buffer or solvent for a specific period of time. This allows labeling of the protein backbone and side-chain amide hydrogens. **3. Proteolytic Digestion:** Digest the protein into smaller peptides using an enzyme such as pepsin or trypsin. This generates peptides that can be separated and analyzed by mass spectrometry. **4. Peptide Analysis:** Inject the peptides into a mass spectrometer, where they are ionized and fragmented.

				Measure the mass-to-charge ratios (m/z) and intensities of the resulting peptide fragments. **5. Data Analysis:** Compare the deuteration levels of the peptides in the protein-ligand complex with those in the control samples (protein alone and ligand alone). Determine the changes in deuteration for each peptide and map them onto the protein structure. **Information Provided:** HDX-MS provides information about the regions of a protein that undergo changes in hydrogen-deuterium exchange upon binding to a ligand. By comparing the deuteration levels of the protein in the complex with the control samples, it is possible to identify the binding site(s) of the ligand and characterize the conformational changes induced by the interaction.
		Size-exclusion chromatography to validate hit binding AI|ffinity & CEITEC MU	Compare elution volumes of protein and protein+hit mixture. If they differ, then the hit is **valid**.	**Principle:** SEC exploits the differences in molecular size and shape to separate biomolecules as they pass through a porous stationary phase (column). Larger molecules elute first because they are not able to enter the pores and thus take a shorter path through the column, while smaller molecules enter the pores and traverse a longer path, resulting in delayed elution. **Experimental Steps:** **1. Sample Application:** Load the sample containing the mixture of biomolecules onto the top of the column. The sample is usually

				prepared in a buffer compatible with the mobile phase. **2. Elution:** Pump a suitable mobile phase (e.g., buffer) through the column, allowing the biomolecules to travel through the stationary phase. **3. Separation:** As the biomolecules travel through the column, they are separated based on their size. Larger molecules pass more rapidly through the column as they are excluded from entering the pores, while smaller molecules spend more time within the pores and elute later. **4. Detection:** Collect fractions or continuously monitor the eluent as it exits the column using various detection techniques, such as UV spectroscopy, refractive index detection, or fluorescence detection. This provides information about the elution profile of the separated biomolecules. **Information Provided:** Information about the size, oligomeric state, and degree of aggregation of sample. Protein-hit complexes will elute slower than the apo form. This information provides implicit confirmation of hit binding to the AR-V7-Cterm.
		NMR-based backbone chemical shifts assignment AI|ffinity & CEITEC MU	Knowing the **assigned backbone** chemical shifts to atoms of AR-V7-Cterm allows us to identify the atoms interacting with the hit (see next task).	Measurement of HNCACB, CBCA(CO)NH, HNCO, and HNCACO NMR spectra of ^15N labeled AR-V7-Ctem apo form, spectra processing (same as for 4D NMR structure determination) and semi-automatic assignment of resonances to backbone atoms by existing commercial-free software.

		Mapping of interactions by 2D-NMR AI|ffinity & CEITEC MU	Measure 2D TROSY-HSQC of each AR-V7-Cterm - hit mixture at a single concentration and overlay with the spectrum of AR-V7-Cterm apo to identify the interacting atoms (**shifted peaks**).	Measurement of TROSY-HSQC NMR spectra of ^15N labeled AR-V7-Ctem - hit mixtures, spectra processing (same as for 4D NMR structure determination) and semi-automatic assignment of resonances to backbone atoms by existing commercial-free software, which is followed by identification of the peaks and the respectives atoms that have been shifted/changed in the protein-ligand mixture. These protein atoms are the ones that interact with the hit compound.
		P-L SS content determination by Circular Dichroism to validate hit binding AI|ffinity & CEITEC MU	Estimate the **(SS) element content** of each AR-V7-Cterm - hit mixture. If it deviates from that of the AR-V7-Cterm apo, it means that the hit is **valid**.	See "1. Receptor Structure'' for experimental details. Assuming that we already have the resonance assignments to 4D NOESY peaks of the AR-V7_C-term apo form, to solve the structure of the AR-V7-Cterm - hit complex we need to measure only the 4D HCNH NOESY spectrum this time. The rest of the procedure will remain the same except that we will use in addition distance restraints obtained from the analysis of photo-CIDNP spectra to accelerated convergence of structure calculations.
	Structural analysis	P-L shape determination by SAXS to validate hit binding AI|ffinity & CEITEC MU	Determine the shape of each AR-V7-Cterm - hit mixture. If it deviates from the shape of AR-V7-Cterm apo, it means that the hit is **valid**.	See "1. Receptor Structure" for experimental details. By comparing the general shape of the AR-V7-Cterm apo from with the shape of the AR-V7-Cterm - hit mixture we can study the conformational changes in the receptor induced by ligand binding.
		Adaptation of the 4D NMR structure determination pipeline to utilize 1D NMR photo-CIDNP data AI|ffinity & NexMR	**Modify** the **4D NMR** structure determination **pipeline to leverage 1D NMR photo-CIDNP data** to accelerate P-L	The 4D NMR structure determination pipeline **update** will consist on the following **steps**: **1. Add new parsing functions** to read 1D NMR photo-CIDNP data of

			structure calculation convergence.	compounds interacting with **flexible proteins** to create structural 1D distance restraints. **2. Overhaul the structural modeling component** to leverage the new 1D distance restraints. **3. Fix** any **bugs** or **limitations** detected during the field use. Once these steps are fulfilled, the new pipeline version will be ready for application and licensing.
		Protein-Hit Structure determination by 4D-NMR or X-ray AI|ffinity & CEITEC MU	Determine the **3D structure** of valid AR-V7-Cterm - hit complexes to enable structure-based drug design.	See "1. Receptor Structure" for experimental details. By comparing the general shape of the AR-V7-Cterm apo from with the shape of the AR-V7-Cterm - hit mixture we can study the conformational changes in the receptor induced by ligand binding.
	Kd measurements	Kd measurements of screened hits (photo-CIDNP, MST, 2D-NMR) AI|ffinity & NexMR & CEITEC MU	**Determined Kd** of all valid hits independently by photo-CIDNP (NexMR), MST (AI|ffinity & CEITEC MU) and 2D TROSY-HSQC by titrating at 6 hit concentrations.	Depending on the specific technique Kd measurements will differ. The general flow looks as below: 1. **Binding Assay Setup:** Prepare a series of protein-ligand mixtures with varying concentrations of the ligand, while keeping the protein concentration constant. Typically, 6 ligand concentrations spanning several orders of magnitude are used. 2. **Quantification of Bound Ligand:** Measure the concentration of the ligand in the bound fraction (protein-ligand complex) 3. **Data Analysis:** Plot the concentration of bound ligands as a function of the free ligand concentration. Use appropriate mathematical models,

				such as the binding equation derived from the law of mass action, to fit the data and determine the Kd value. Nonlinear regression analysis is commonly employed for this purpose. 4. **Kd Calculation:** The Kd value represents the ligand concentration at which half of the protein binding sites are occupied. It can be directly determined from the fitted curve or calculated from the equilibrium dissociation constant equation.
	Compound synthesis	Purification and analysis of compounds AnHorn Medicines	Synthesize **10-15** potential small molecules based on the results of the Kd values	Once the newly synthesized compound is successfully obtained, it needs to be appropriately purified.
		Verification of hit compound structure AnHorn Medicines	Verification of **10-15** potential small molecules for in vitro assay	Chemists also need to develop and establish efficient analysis platforms and methods using LC-MS, NMR, and HPLC to confirm the accuracy of the compound's structure.
	In vitro experiments	Growth inhibition assessment of hits on prostate cancer cells AnHorn Medicines	Test the inhibitory effect on cancer cell growth of the **10-15** selected small molecules	In this experiment, we aim to confirm whether structurally modified inhibitors can effectively suppress tumor cell proliferation. The experimental procedure involves seeding human prostate cancer cells into a 96-well plate and incubating them at 37°C with 5% CO2 for one day. Afterward, the tested drugs, which have been diluted in a series, are added to the culture medium and incubated for 5-7 days. MTS reagent is then added to the wells, and the plate is incubated at 37°C for 1 hour. Subsequently, the optical density (OD) at 490 nm is measured using a

				full-spectrum plate reader to calculate the concentration of the drug that causes a 50% inhibition of prostate cancer cell proliferation, known as the IC50 value.
2.3 Hit to Lead (weight: 35%) Executing company : AnHorn Medicines (weight: **70** %) AI|ffinity & NexMR & CEITEC MU (weight: **30** %)	NMR-AI & in silico-based design	Hit structure optimization by in silico method AnHorn Medicines	Obtain **10-20** novel small molecules based on the optimization results	Based on the preliminary Hits obtained from the previous stage and the co-crystal structure with AR-DBD, we will utilize the module within our internal drug platform to perform compound structure modifications according to the defined binding pocket features. Subsequently, we will re-dock the modified compounds to predict their binding affinities.
		Adaptation of the deepScaffOpt to utilize 1D NMR photo-CIDNP data AI|ffinity & NexMR	**Modify deepScaffOpt to accept 1D NMR photo-CIDNP data** for model training and enable both ligand- and structure-based drug design with higher accuracy	The deepScaffOpt update will consist on the following steps: **1. Add new parsing functions** to read 1D NMR photo-CIDNP data of compounds interacting with flexible proteins. **2. Overhaul the core AI functions** to leverage the information of the new photo-CIDNP data. **3. Fix** any **bugs** or **limitations** detected during the field use. Once these steps are fulfilled, the new deepScaffOpt version will be ready for application and licensing.
		Hit structure optimization by AI in incremental feedback loops (multiple rounds) AI|ffinity & NexMR	Train deepScaffOpt using hits with measured Kd. Create in silico analog library (**modified hits**) from the strongest hits. Score analog library with deepScaffOpt and select **5-10** molecules for synthesis and Kd measurement.	First hundreds of analogs will be generated from selected hit compounds using third party DL-based de novo drug design code, by simultaneously optimizing properties like synthetic accessibility, solubility, and cytotoxicity. Then a deepScaffOpt model will be trained with specially designed feature vectors (2D

				molecular representations) that contain structural information derived from the analysis of photo-CIDNP spectra. This model will be used to score and sort the analogs by predicted binding affinity. Finally, selected top-scored analogs will be selected for synthesis by AnHorn or ordered from vendors. This procedure will be repeated in incremental feedback loops, each time selecting new molecules for experimental validation, solving their 3D structure in complex with the receptor and retraining deepHitMiner. Optionally, once we obtain the first 3D structures of P-L complexes we will shift to structure-based drug design with 3D pharmacophore feature vectors encompassing structural information from photo-CIDNP data.
	Binding validation and binding site characterization	Mapping of interactions by 2D-NMR AI|ffinity & CEITEC MU	Measure 2D TROSY-HSQC of each AR-V7-Cterm - binder mixture at a single concentration and overlay with the spectrum of AR-V7-Cterm apo to identify the interacting atoms **(shifted peaks)**.	Measurement of TROSY-HSQC NMR spectra of ^15N labeled AR-V7-Ctem - binder mixtures, spectra processing (same as for 4D NMR structure determination) and semi-automatic assignment of resonances to backbone atoms by existing commercial-free software, which is followed by identification of the peaks and the respectives atoms that have been shifted/changed in the protein-ligand mixture. These protein atoms are the ones that interact with the hit compound.

	Structural analysis	Protein-Lead Structure determination by 4D-NMR or X-ray AI|ffinity & CEITEC MU	Determine the **3D structure** of each AR-V7-Cterm - binder complexes to enable structure-based optimization.	See "1. Receptor Structure" for experimental details. By comparing the general shape of the AR-V7-Cterm apo from with the shape of the AR-V7-Cterm - binder mixture we can study the conformational changes in the receptor induced by ligand binding.
	Kd measurements	Kd measurement of modified hits (photo-CIDNP, MST, 2D-NMR) AI|ffinity & NexMR & CEITEC MU	Measure Kd of **5-10** synthesized **modified hits** (as above). Use them to retrain deepScaffOpt and **repeat the "Hit structure optimization by AI"** task until molecules with nanomolar Kd (**leads**) are obtained.	Same as in "2.2 Hit Validation".
	Compound synthesis	Purification and analysis of compounds AnHorn Medicines	Synthesize **5-10** potential small molecules based on the results of the Kd values	Same as in "2.2 Hit Validation".
		Verification of optimized compounds AnHorn Medicines	Verification of **5-10** potential small molecules for in vitro assay	Same as in "2.2 Hit Validation".
	In vitro experiments	Growth inhibition assessment of leads on prostate cancer cells AnHorn Medicines	Test the inhibitory effect on cancer cell growth of the **5-10** selected small molecules	Same as in "2.2 Hit Validation".
Development of PROTACs for AR-V7 [weight: 15%]				
AR-V7 PROTAC: Hit identification & validation Executing company : AnHorn Medicines (weight: **100** %)	In silico-based design	AR-V7 PROTAC structure design AnHorn Medicines	Design **10–20** AR-V7 protein PROTACs based on in silico methods	To design the potential compounds as PROTACs, it is necessary to obtain the binding conformation of AR-V7 DBD with the ubiquitin ligase E3. Therefore, researchers at AnHorn Medicines will perform protein-protein docking experiments. All possible binding modes between the two proteins, obtained through rotation and translation, will be evaluated using energy-based scoring functions to predict the

				feasibility of each binding mode. The binding conformation with the highest score will be selected as the potential binding site for designing AR-V7 PROTACs.
	B1b. Compound design and synthesis	Purification and Verification of designed compounds AnHorn Medicines	Synthesize **5-10** potential PROTACs based on the results of the predicted binding affinity	Same as in "2.2 Hit Validation".
	B1c. In vitro experiments	Degradation assay on AR-FL/AR-V7 protein AnHorn Medicines	Test the degradation ability of **5-10** selected PROTACs on AR-FL/AR-V7 protein	In this experiment, the efficacy of PROTAC in effectively degrading the target protein is used as an indicator of drug activity. The experimental procedure involves seeding human prostate cancer cells into a 12-well plate and incubating them at 37°C with 5% CO2 for one day. Afterward, the tested drugs, which have been diluted in a series, are added to the culture medium for 0.5-24 hours. Subsequently, the cells are lysed, and the total protein is extracted. Western blotting is then performed to detect the protein levels of the androgen receptor, enabling the calculation of the concentration of the drug that causes a 50% degradation of the androgen receptor, known as the DC50 value.