Vrije Universiteit Drug Discovery Center
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DDC News No. 1

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The VU Drug Discovery Center

The Drug Discovery Center (DDC) is an interfaculty research center, which integrates various disciplines in the area of drug discovery. Expert knowledge from the faculties of Earth and Life Sciences (FALW), Natural Sciences (FEW) and the VU University Medical Center (VUmc) is combined in various interdisciplinary programs.

The DDC acts as a specific incubator for drug discovery projects that may lead to high value intellectual property rights. As such, the DDC is dedicated to the development and commercialization of specific products/technologies.

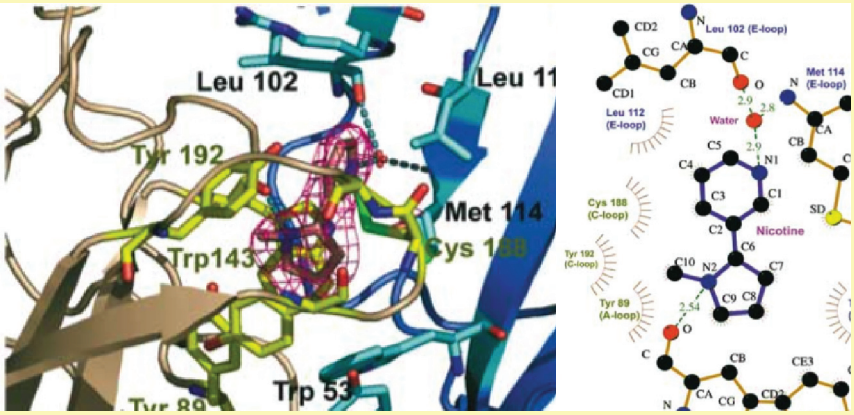
The Drug Discovery Center was founded in November 2006 by Prof. Dr. H. Irth (Dpt of Biomolecular Analysis, FEW), Prof. Dr. R. Leurs (Dpt of Medicinal Chemistry, FEW) and Prof. Dr. A.B. Smit (Dpt of Molecular and Cellular Neurobiology, FEW)

Newly appointed VU-DDC professor with focus on signalling networks

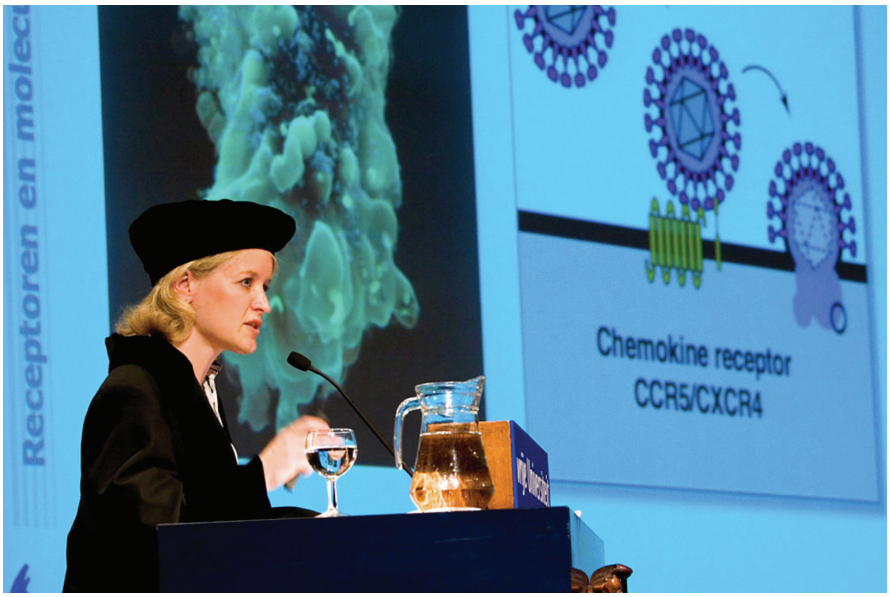
Prof. Dr. Martine Smit accepted her chair "Receptors and Molecular Communication" by giving a public inaugural lecture entitled "Interactions that matter: from genes to medicines". In her speech Prof. Smit highlighted the importance of the family of G-protein coupled receptor in cellular (mis)communication and the recent insights on viral piracy of this essential gene family. She illustrated with recent unpublished work of her research group, that unravelling the cellular signalling networks, activated by viral G-protein coupled receptors, can be a great benefit for designing potential therapeutic interventions. In the coming years, prof. Smit will focus on the signalling networks activated by various viral and human chemokine receptors in close collaboration with Dr. Marco Siderius, Dr. Iwan de Esch and prof. dr. Rob Leurs. Prof. Smit was appointed in November 2005 as one of the two Fenna Diemer Lindeboom chairs in the Faculty of Science.

Drug discovery program: Nicotinic acetylcholine receptors.

The first VU-DDC funded interdisciplinary program started in 2006 and is on the use of the acetylcholine binding protein (AChBP) with the aim to enable fragment and structure-based drug design for various types of nicotinic acetylcholine receptors. The program uniquely uses the acetylcholine binding protein (AChBP) of which the structure with and without ligands bound has been solved (see inset) and which is a mimic of the extracellular ligand-binding domain of the nAChRs. This VU-DDC program crucially brings together researchers from the different disciplines. Protein engineering is used for constructing human ligand binding sites within the AChBP (Smit). Computational modeling and organic chemistry are being used to generate ligands with receptor subtype specificity (De Esch), whereas X-ray crystallography is used to identify in detail ligand-receptor interactions at atomic resolution (Sixma). Novel MS methodology is used to implemented fast screening methods identifying compounds, which interact with the AChBP binding site (Irth). In this program 10 scientists have joined forces to make progress experimentally and in generating novel concepts.



Nicotine binding to AChBP. Left: The nicotine binding site shown in stereo, ligand in ball-and-stick, with electron density superimposed, and residues in the binding site in yellowish (principal side) and blueish (complementary side). Right: Schematic ligand interactions of nicotine showing hydrogen bonds and van der Waal contacts. (Celie et al. (2004) Neuron 41, 907-914).



Prof. Martine Smit during her inaugural speech

Research Highlights

VU-DDC Fragment-Based Drug Discovery platform is rapidly expanding.

Dr. Iwan de Esch
Division of Medicinal Chemistry

Fragment-Based Drug Discovery (FBDD) is emerging as a design intensive and efficient approach to develop new medicines. VU-DDC has set up an extensive FBDD platform.

Many pharmaceutical companies try to find novel hits for the emerging drug targets by unleashing high-throughput screening technologies. Large collections of compounds are randomly screened for ligands that interact with the target. A major problem is that HTS methods find compounds that have a molecular weight between 400 and 700 Da and often violate many of Lipinski's "rules" for druglikeness. Typically, lead-like compounds become larger, more complex and more lipophilic during the optimization stages in which both the affinity and specificity of the lead compound have to be increased. In this process, HTS-derived compounds move further away from the characteristics that define a good drug. Thus, HTS starts by using a non-ideal pool of compounds. It is also becoming clear that ligands that are being developed with HTS often have a selectivity profile that is difficult to modulate. Many derivatives have to be synthesized before a clinical candidate with the right selectivity profile has been identified. HTS is considered a resource intensive drug discovery approach as more than hundreds of thousands of compounds have to be screened and many thousands of compounds have to be synthesized to obtain a clinical candidate.

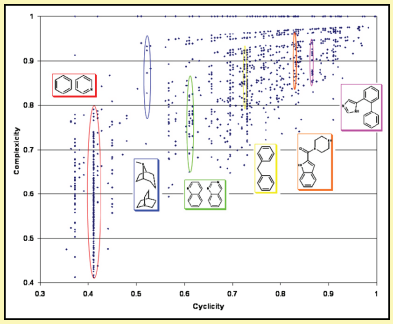
In contrast, FBDD is emerging as a design intensive drug discovery approach. The essence of FBDD is to get an early (experimental) confidence in the design of new ligands. Lead compounds are developed in a rational and stepwise manner. For this, small molecular weight compounds (so-called fragments) that interact with the biological targets have to be identified. Because of the limited size of the fragments (typical molecular weight is between 150 and 300 Da) the corresponding chemical space is significantly smaller than for the bigger drug-like ligands. Sampling of chemical space using fragments actually can obtain reasonable coverage, and most often the first round of screening is performed with a library of around 1000 fragments.

The low molecular weight fragments generally have moderate to low affinity for a protein target (typically 10µM-10mM) and biophysical screening technologies are used to characterize the weak interactions. Binding fragments can be grown (stepwise) into drug-like molecules by iterative cycles of design, synthesis and screening. This optimization is guided by structural protein information that is obtained either experimentally (X-ray, NMR) or by molecular modeling studies. As such, FBDD belongs to the Structure-Based Drug Design approaches. It is becoming clear that FBDD is very efficient. Not only is it possible to obtain very potent compounds quickly, it is also suggested that it is easier to obtain a fragment with a beneficial selectivity profile and maintain this profile while growing the compound into a drug than it is to identify a drug-like high affinity compound and subsequently try to modify the selectivity profile in this late stage.

VU-DDC has set up an extensive FBDD platform. Drug discovery efforts are underway for several targets (including kinases, AChBP and ligand-gated ion channels and GPCRs) using a variety of screening technologies. For example, a fragment-based screening protocol using Mass Spectroscopy (MS) is currently being set up by Prof. Irth and Dr. Koolen. Furthermore, Surface Plasmon Resonance (SPR and X-ray analysis (collaboration with Prof. Titia Sixma, NKI) are explored.

Within the TI Pharma financed Ligand-Gated Ion Channels project (total 2.5 M€), X-ray and SPR screening are foreseen. This structure-based drug discovery program has been ongoing in collaboration with the group of Prof. Titia Sixma of the NKI for several years and continues to be very successful.

Gerdien de Kloe has in the first three months of her PhD studies analyzed and optimized the VU-DDC fragment library that consists of 1200 proprietary compounds. The quality of the library is validated by analysis of the physicochemical profile and scaffold distribution and by diversity analysis using a set of Drug-Like Index Descriptors. The library will be an ideal premise for use in the FBDD platform



Scaffold distribution of the VU-DDC fragment library reveals excellent coverage of chemical space.

Financed in part by TI Pharma, the VU-DDC has obtained the most sensitive surface-plasmon-resonance (SPR) equipment to date. The Biacore T100 with a list price of Euro 420.000 is able to detect "label-free" the interaction of ligands to their immobilized biological targets.

The Biacore T100 is latest addition to the high-tech VU-DDC infrastructure.

The latest addition to the FBDD platform is a STW funded project (total 1.1 M€) that focuses on efficient development of tyrosine kinase inhibitors that are considered promising therapeutics for the treatment of skin cancer and prostate cancer. The project is a close collaboration between several academic groups and small biotech companies:

The group of Dr. Cees Tensen (Department of Dermatology, Leiden University Medical Center, LUMC) will be responsible for protein production, in vitro kinase assays and the development and implementation of cell- and animal-based inhibitor assays. The group of Dr Gregg Siegal (Leiden Institute of Chemistry, LIC) will be responsible for carrying out the NMR-based fragment screening using the proprietary "Target-Immobilized NMR Screening" (TINS) technology. This group will also perform 3D structure determination of kinase-ligand complexes using NMR analysis.

During the recent LACDR spring symposium 2007, VU-DDC PhD student Atilla Akdemir won a price for best oral presentation. Atilla described his efforts to develop efficient in silico screening protocols to identify potent ligands for AcetylCholine Binding Protein (AChBP) and the human α7 Nicotinic Acetylcholine Receptor (nAChR). During the same conference, PhD student Ewald Edink won the best poster award for his work on the structure-based design and synthesis of new ligands for AChBP (both projects in collaboration with the group of Prof. T. Sixma, NKI).

Price winners Akdemir (left) and Edink (right)

The Medicinal Chemistry Group of the VU-DDC (De Esch and Leurs) will convert the generated data into the design and synthesis of the actual kinase inhibitors.

Next to the three academic groups, the research consortium consists of four innovative biotech companies. Pyxis-Discovery offers chemistry-based drug discovery services such as novel screening libraries. Zobio provides the project with expertise in handling NMR screening data. KeyDP is able to efficiently obtain X-ray analysis of the target protein and of the protein-ligand complexes. Finally, Pamgene´s 3D Microarray Technology will enable thorough kinase profiling. By testing kinase activity on a large panel of substrates in the presence of the ligands under development, a real-time kinetic read-out can be obtained that enables assessment of IC50 data and selectivity profiles for a range of different kinases.

It is anticipated that short iterative cycles of design, synthesis, biophysical screening and pharmacological evaluation will result in potent tyrosine kinase inhibitors. The project will cover nearly the entire scope of preclinical drug development. The members of the research consortium are believed to provide a highly complementary set of skills and are not only eager to validate novel drug discovery technologies but also aim to obtain novel and patentable lead compounds for tyrosine kinase receptors. For the VU-DDC, this project will be a valuable extension of the FBDD platform.

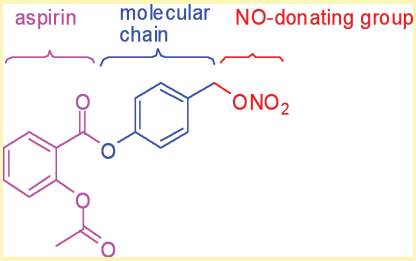
Cancer tied to a chain
Surprising finding sheds light on a promising anti-cancer drug.

Prof. Rob Leurs
Division of Medicinal Chemistry

An intensively studied hybrid drug with a rising status in the fight against cancer turns out to have a completely different mode of action than had been initiatially anticipated. It kills animal and human cancer cells not through the synergistic interaction between the two medical components aspirin and nitric oxide, but simply through the 'molecular chain' that connects them. This finding is revealed from studies carried out by DDC scientists Niels Hulsman and Maikel Wijtmans and colleagues from the Department of Pharmacochemistry at the Vrije Universiteit Amsterdam and from the Department of Experimental Oncology & Radiobiology at the University of Amsterdam. The findings do not only offer detailed insights into the anti-tumor action of the hybrid drug, but also show convincingly that a hybrid drug does not necessarily exerts its biological effects through a combined action of both medical components.

The results have been published in the Journal of Medicinal Chemistry (J Med Chem. 2007 May 17;50(10):2424-31). The DDC researchers stripped the hybrid drug, called nitric oxide-donating aspirin, from both its components nitric oxide or aspirin and found that this did not reduce the high anti-tumor action. Much to their surprise, the molecular chain alone still effectively killed cancer cells, despite the fact that by design this chain only serves to passively connect the two components. In fact, the activity of the molecular chain alone was about 10 times higher than the parent hybrid drug.

Further studies by the DDC scientists showed that the molecular chain undergoes an efficient chemical reaction with glutathione through an intermediate quinone methide. Without this essential guard-molecule glutathione, the cancer cells become extremely vulnerable and quickly meet their demise. These specific structural characteristics of the molecular chain may aid in the inception of novel anti-cancer medicines.



The chemical structure of nitric oxide-donating aspirin

Important publications

Lim HD, Smits RA, Bakker RA, van Dam CM, de Esch IJ, Leurs R., Discovery of S-(2-guanidylethyl)-isothiourea (VUF 8430) as a potent nonimidazole histamine H4 receptor agonist, J Med Chem. 2006 Nov 16;49(23):6650-1.

Bruyneel B, Hoos JS, Smoluch MT, Lingeman H, Niessen WM, Irth H., Trace analysis of proteins using postseparation solution-phase digestion and electrospray mass spectrometric detection of marker peptides, Anal Chem. 79 (2007 1591.

van Nierop, P., Bertrand, S., Munno, D. W., Gouwenberg, Y., van Minnen, J., Spafford, J. D., Syed, N. I., Bertrand, D., and Smit, A. B. Identification and functional expression of a family of nicotinic acetylcholine receptor subunits in the central nervous system of the mollusc Lymnaea stagnalis. J Biol Chem 281 (2006) 1680-1691.

Vacancies

DESIGN & SYNTHESIS
(Dr. Iwan de Esch, far@few.vu.nl)

1. Post-Doc position (3y, STW)
Fragment-based drug discovery (FBDD) of tyrosine kinase inhibitors
The applicant will be responsible for converting structural, biophysical and pharmacological data into the design and synthesis of novel ligands. Experience in medicinal or synthetic organic chemistry is required, as is an interest in rational drug discovery approaches.

2. PhD position (4y, TI Pharma)
Modeling ligand-GPCR interactions
The applicant will be responsible for combining GPCR-related cheminformatic and bioinformatic data to come to a more detailed understanding of ligand-receptor interaction. A strong background in QSAR, computational chemistry, pharmacophore modelling, in silico screening, and ligand-based design is required. The ability to work well with an interdisciplinary team is a must.

3. Post-Doc position (3y, TI Pharma)
Modeling GPCR receptor modulation
The applicant will be responsible for developing accurate structural models for GPCRs. By working in close collaboration with the experimental groups (e.g., molecular biology, pharmacology and the synthetic chemistry groups) we aim to come to an improved understanding of ligand-receptor interaction and receptor modulation. The candidate should have a PhD in Chemistry, or Biophysics and ample experience in a variety of computational chemistry techniques e.g., homology modeling, docking, MD and QM.

4. Technician (3y, TI Pharma)
Synthesis of biologically active compounds (hit and lead optimisation)
The applicant will be responsible for the synthesis of biologically active compounds for our ongoing drug development efforts. The candidate should have a degree in Synthetic Organic Chemistry and experience in a broad repertoire of organic transformations as well as compound characterization using various analytical methods (e.g., IR, NMR, HRMS, LCMS).

5. Post-Doc position (2 years, VU)
Design and Synthesis of GPCR ligands
The applicant will be responsible for the drug discovery efforts of an undisclosed GPCR target. The candidate should have a degree in Medicinal Chemistry or Synthetic Organic
Chemistry. The position requires a strong teamplayer with profound knowledge of organic chemistry.

MOLECULAR PHARMACOLOGY
(Prof. Martine Smit, far@few.vu.nl)

6. Post-Doc position (3y, VU)
Implementation of SPR technology
The applicant will be responsible for implementing SPR technology for ongoing drug discovery efforts. Expertise in the preparation of proteins for SPR measurements, SPR characterization of ligand-protein interaction, implementation of fragment binding studies and hit optimization efforts and comparison of SPR data with other biophysical data. Ideally, the candidate has a PhD in Biochemistr or Medicinal Chemistry.

7. Post-Doc position (3y, TI Pharma)
Receptor (de)activation of GPCRs
The applicant will focus on (de)activation mechanisms of GPCRs using (microscopic) fluorescent technologies. Constitutive and ligand-induced signalling will be analyzed using classical (FRET/BRET) and multiplex High-Content Screening (HCS) techniques. Ideally, the candidate has a PhD in Biochemistry, Cell biology or Pharmacology and ample experience with fluorescent technologies.

8. PhD position (4y, TI Pharma)
Receptor dimerisation
The applicant will study heterodimerization of GPCRs and examine the pharmacological implications of agonist/antagonists binding to the GPCR heterodimers. The candidate should have a strong background in receptor pharmacology or biochemistry.

9. Post-Doc position (2y, TI Pharma)
Biophysical/biochemical GPCR studies
The applicant will first focus on the purification of GPCRs, followed by the biophysical analysis of purified GPCRs to generate structural information on GPCRs. Ideally, the candidate has a PhD in Biochemistry or Biophysics and ample experience with purification of membrane-bound receptors.

10. Post-Doc position (2y, TI Pharma)
Targeting of GPCRs
The applicant will be responsible for setting up of screening assays for the identification of ligands targeting GPCRs/GPCR dimers. The candidate should have a PhD in Pharmacology, Biochemistry, Cell biology or Medicinal Chemistry and ample experience with GPCR-based assays.

BIOMOLECULAR ANALYSIS
(prof. Hubertus Irth, irth@few.vu.nl)

11. PhD position (4y, TI Pharma)
Fast analysis of metabolites and their interaction with drug targets
(in collaboration with Prof. Nico Vermeulen, Molecular Toxicology)
In this project the primary aim is to develop novel LC-MS based analytical methodologies for the rapid chemical analysis of metabolic mixtures in cellular systems. The ambitious candidate should have a background and experience in the following areas: HPLC-MS of small molecules and automated sample pretreatment techniques. Knowledge of drug metabolism is appreciated.

MOLECULAR AND CELLULAR NEUROBIOLOGY (Prof. Guus Smit, guus.smit@falw.vu.nl)

12. PhD Position (4y, TI Pharma)
Constructing ligand-binding domains of nACh receptor subtypes
This project aims at generating and studying engineered ligand binding sites of human nicotinic acetylcholine receptors in order to embark on fragment and structure-based drug design efforts for subtypes of this pharmacologically important class of receptors. The candidate should have a background in molecular biology/ biochemistry and experience with computational modeling.