RSC CICAG/RSC BMCS 20 Years of the Rule of Five Meeting, Sygnature Discovery, BioCity, Nottingham, UK.

It has been over twenty years since Lipinski published his work determining the properties of drug molecules associated with good solubility and permeability [1]. The “Rule of 5” rapidly became synonymous with drug-like properties though-out the Pharma industry (16,000 citations). Since then, there have been a number of additions and expansions to these “rules”. There has also been keen interest in the application of these guidelines in the drug discovery process and how these apply to new emerging chemical structures “beyond the rule of five” (bro5) such as macrocycles.

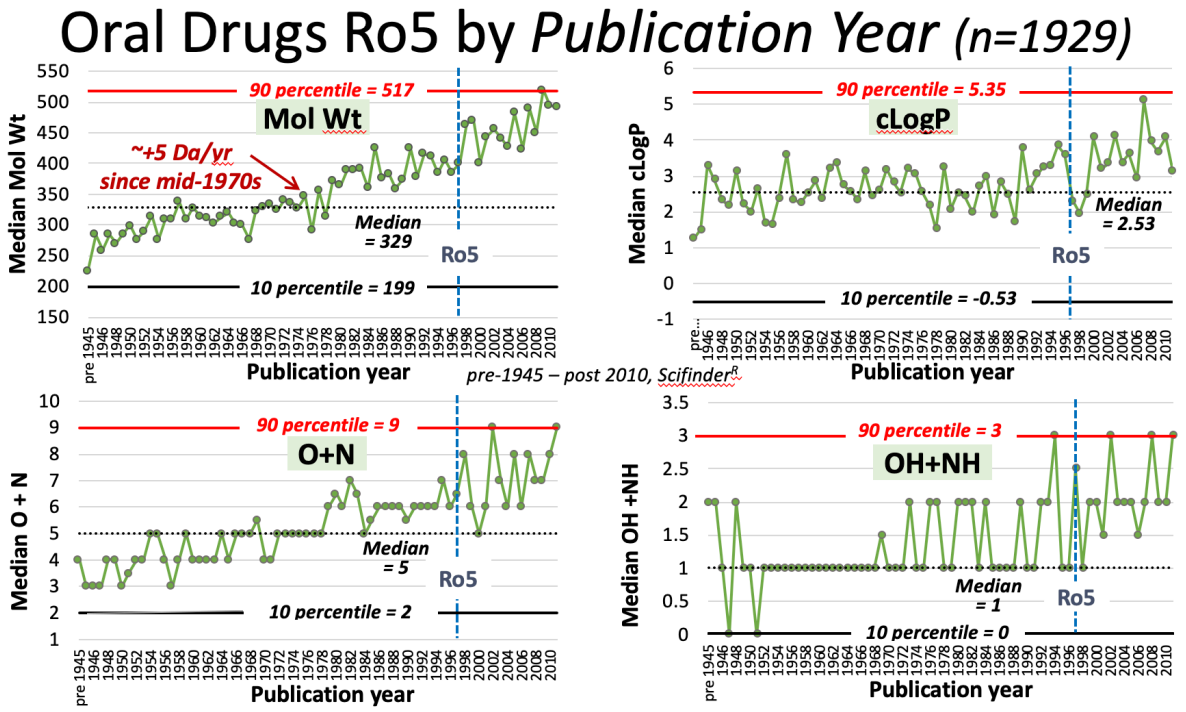
The 20 Years of the Rule of Five Meeting brought together researchers from a number of different areas of drug discovery and provided both a historical overview of the use of Lipinski’s rules, as well as looking to the future and how these rules might evolve in the changing drug compound landscape. The meeting had a capacity attendance of over 100, with Sygnature kindly providing the venue. The audience was a nice mix of industry “veterans”, students and those new to the industry. The meeting format was a morning session giving a historical viewpoint followed by a panel discussion, and the afternoon was dedicated by a more forward looking session again followed by a panel discussion.

Paul Leeson (Paul Leeson Consulting) started the meeting by reminding everyone that the original paper came about as a response to hits from high-throughput screening that had unfavourable physicochemical properties.

Experimental and computational approaches to estimate solubility and permeability in discovery and development settings are described. In the discovery setting `the rule of 5' predicts that poor absorption or permeation is more likely when there are more than 5 H-bond donors, 10 H-bond acceptors, the molecular weight (MWT) is greater than 500 and the calculated Log P (CLogP) is greater than 5 (or MlogP>4.15).

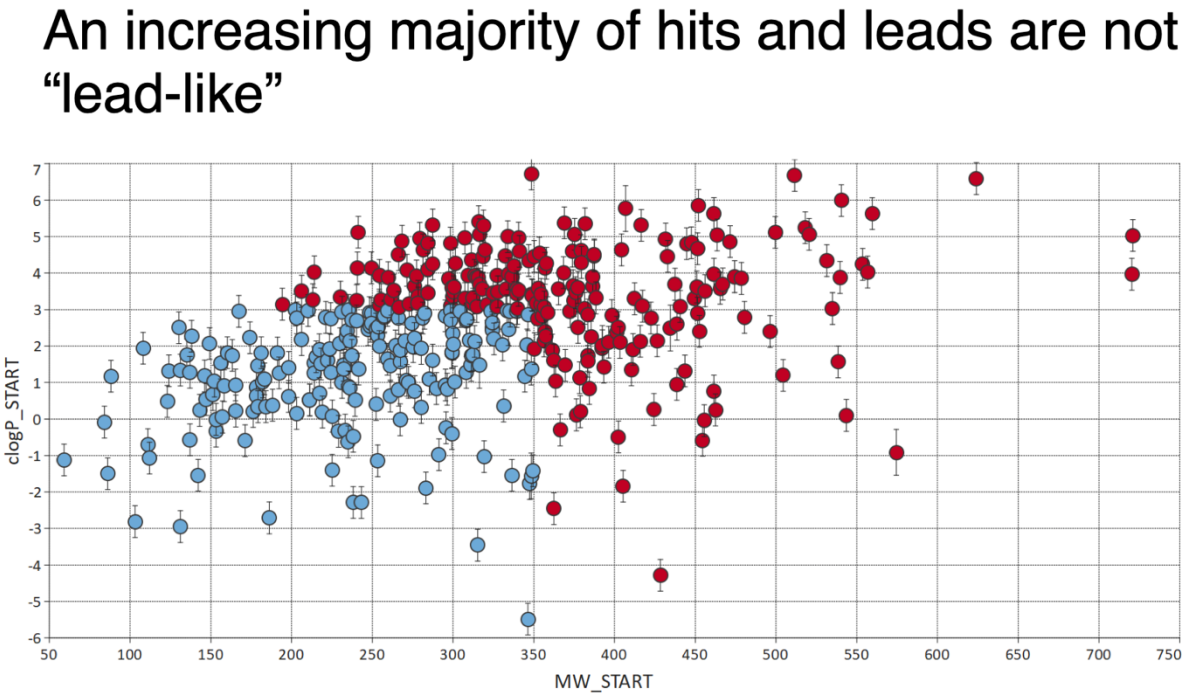
The calculated properties can be categorised as “Size, lipophilicity and polarity”, whilst molecular weight is used to describe size, perhaps this overestimates the contributions of some atom e.g. Fluorine. Calculated LogP is used as a measure of lipophilicity but it was pointed out that different algorithms may give different results. The number of H-bond donors and acceptors in the original paper is actually a count of O and N or OH and NH and not a true measure of all atoms and their ability to form hydrogen bonds. In the panel discussion it was suggested that the use of the use of H-bonds scales such as those proposed by Abraham [2] might be interesting.

Looking at the properties of published drugs (we of course don’t know about all failures) since the publication of the rule of 5 paper, Molecular weight has continued to increase, cLogP appears to have plateaued around 4, whilst there is an increase in the number of HBA, there is only a marginal increase in HBD.



It was suggested, and later elaborated in the discussions that much of the increase is probably driven by the targets now being addressed, that have extended and/or shallow binding sites, protein-protein interactions, HIV, HCV. It was noted that many of the high molecular weight drugs had extremely high affinity (sub-nanomolar) and thus required low doses.

Michael Shultz (Novartis) continued the review of properties used to predict “drug-likeness” introducing polar surface area (TPSA), rotatable bonds (RotB), fraction of sp3 carbons (Fsp3) and Aromatic ring counts (#ArRNG). He also highlighted the difference between, hits, leads and drugs, and suggestion that lead-like structures have MWt <350 and cLogP <3. He also noted that historically in drug discovery optimisation process there are significant increases to MWt and cLogP [3]. However, using data from a recent publication [4] he suggested that leads are becoming less lead-like with increasing MWt and cLogP in particular. In fact, only 47% of recent leads have MWt <350 and cLogP <3. This was followed up in the panel discussion with the suggestion that a good strategy might be to first prune down any hits from screening rather than seeking to immediately add to the hit structure.

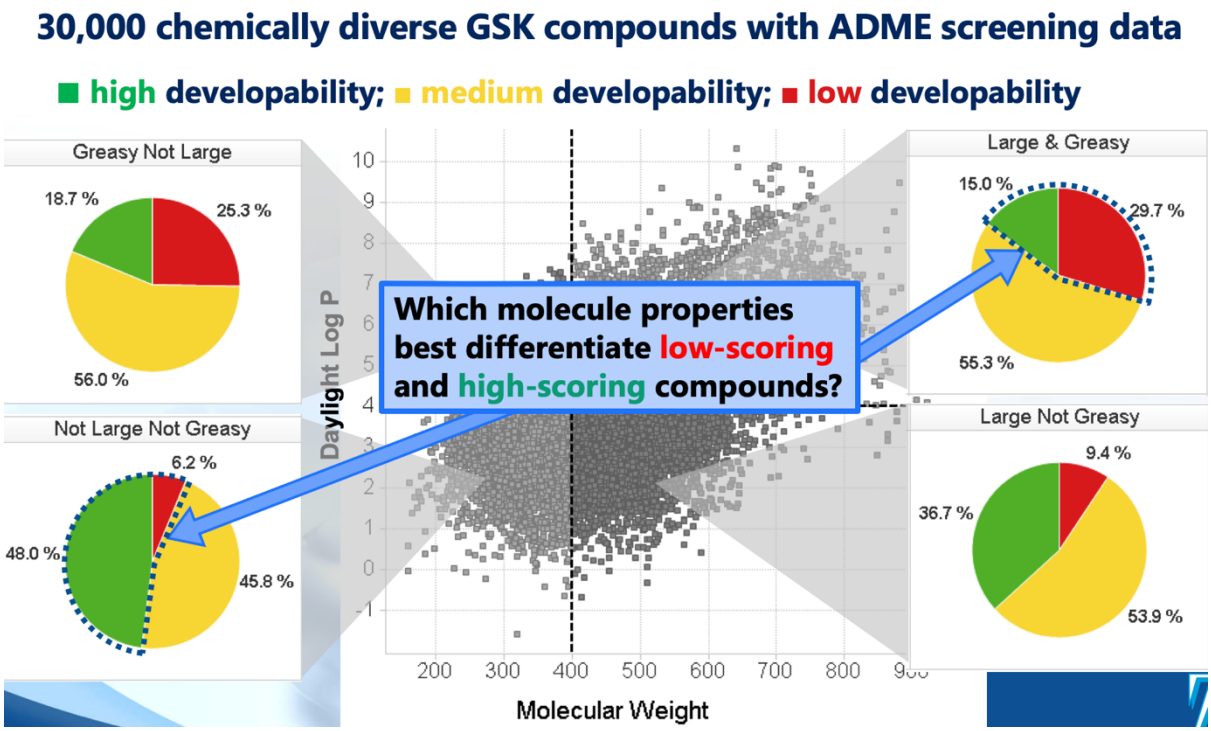


Michael also highlighted the issues of experimental measurement of LogP and solubility of highly lipophilic (>6) and poorly water-soluble compounds.

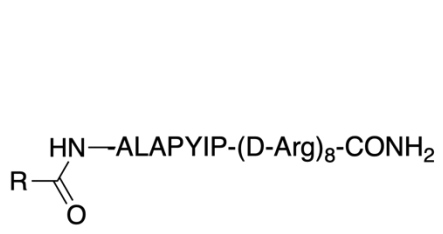
Tim Ritchie (Zerlavanz Consulting) continued the discussion on descriptors focussing on aromatic and aliphatic descriptors and the impact on ‘developability’ [4,5]. Increasing carboaromatic ring count decreases solubility, and in addition leads to increased plasma protein binding, increased CYP450 inhibition and HERG issues. Replacing carboaromatic rings with heteroaromatic or heteroaliphatic rings can lead to improvements in all properties. Interestingly whilst MWt and cLogP have increased, the aromatic-aliphatic atom balance has not changed in oral drugs over time.

Tim also described the Developability score [7] four distinct cLog P/molecular weight regions that define optimal and sub-optimal chemical space, and a developability score derived from regression models using solubility, permeability, protein binding and 3A4 inhibition screening data. Whilst the sector MWt <400, cLogP <4 suggested

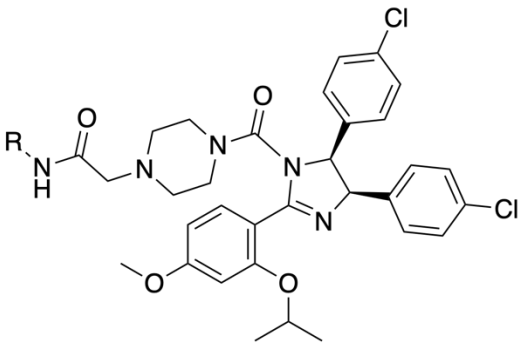
the greatest chance of success, it was noted that even the MWt >400, cLogP >4 sector included some developable molecules albeit at a much lower chance of success.



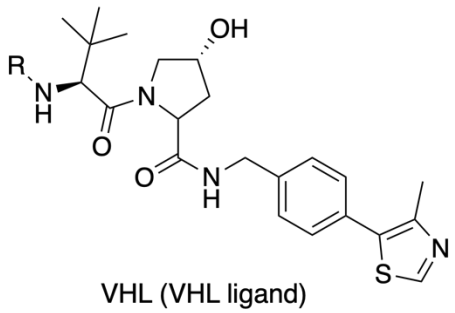
John Priestner (GSK, Univ of Strathclyde) described their work investigating modifications to the linker functionality of BET PROTACs and the effect on physicochemical properties. PROTACs [8] are bifunctional molecules that bind to the target protein and an E3 ligase, the simultaneous PROTAC binding of two proteins brings the target protein in close enough proximity for polyubiquitination by the E2 enzyme associated to the E3 ligase, which flags the target protein for degradation through the proteasome. Despite having properties outside the rule of 5 the first PROTAC entered phase I clinical trials in 2019. Whilst the first PROTACs used peptidic E3 ligase ligands, more recent PROTACs use small molecule ligands.



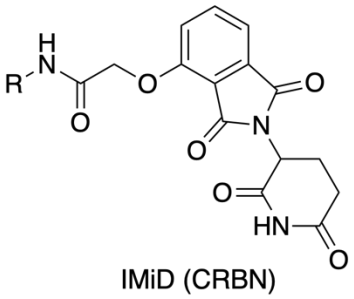
HIF-1α peptide (VHL ligand)



Nutilin (MDM2)

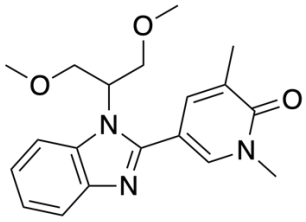


VHL (VHL ligand)



IMiD (CRBN)

In this work the VHL ligand was chosen, and a pan-BET ligand (GSK001) identified from an encoded library screen.

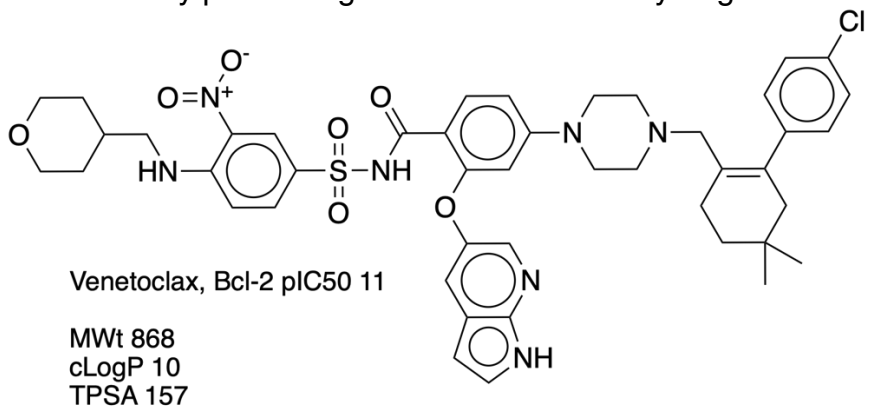


GSK0001

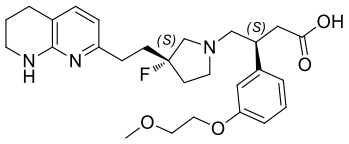
A variety of synthetic strategies were investigated for linker incorporation, and amide coupling proved to be the most robust. The physicochemical properties of the resulting PROTACs were determined and shown to cover a wide area of property space. Several examples have been shown to degrade the BDR4 in vitro. A more detailed investigation of linker properties is ongoing.

Elisabetta Chiarparin (AZ) described their work investigating the impact of conformational constraints on drug-like properties. In a series of elegant NMR studies they showed that macrocycle formation can preorganise ligands into the bioactive conformation leading to significant potency increases. The NMR studies also highlight reduced flexibility (NMR Rot lower), but also masking of hydrogen bond donor/acceptors. This polarity masking lead to exposed polar surface area (ePSA) descriptor which correlates with Caco-2 permeability. Elisabetta then made the case that we need to use 3D descriptors (3D Ro5) to better describe the properties of drugs, particularly high molecular weight drugs. In the case of Venetoclax despite very high molecular weight, cLogP and TPSA it has acceptable

oral bioavailability. Conformational analysis and NMR studies suggest it adopts a folded structure driven by pi-stacking and intramolecular hydrogen bonds.



James Thompson (GSK) reported their work Investigating the Chameleonic Properties of $\alpha_V\beta_6$ Integrin Antagonists for the Treatment of IPF. The binding site is highly polar, and the two key binding interactions are salt bridges to a metal ion an aspartic acid residue in the receptor. Small molecule ligands for RGD integrins are zwitterions containing polar acidic and basic functionality [9].



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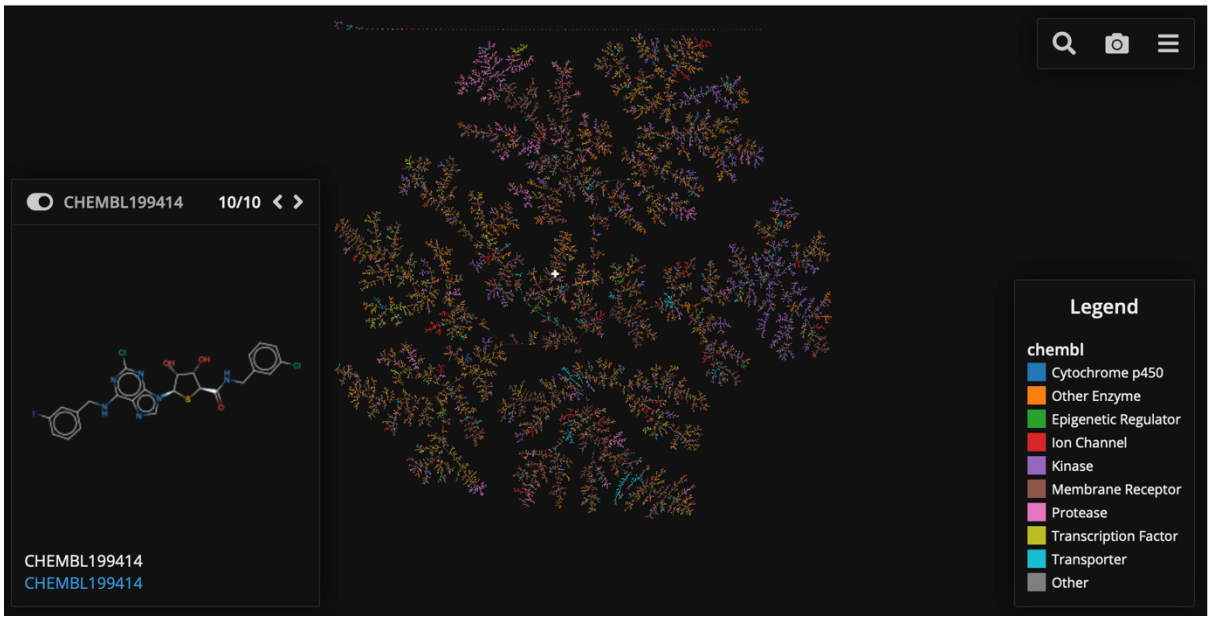
Interestingly diastereomer **1** was found to be more permeable than the oother diastereomers despite identical pK_a and LogD. It was hypothesised that this was due to an intramolecular hydrogen bond between the acid and the tetrahydro-1,8-naphthyridine. A series of analogues were prepared and ^{15}N NMR used to investigate conformations and presence of the intramolecular hydrogen bond and the impact on permeability. These methods have subsequently been successfully used to rationalise the permeability of several more recent series of $\alpha_V\beta_6$ antagonists.

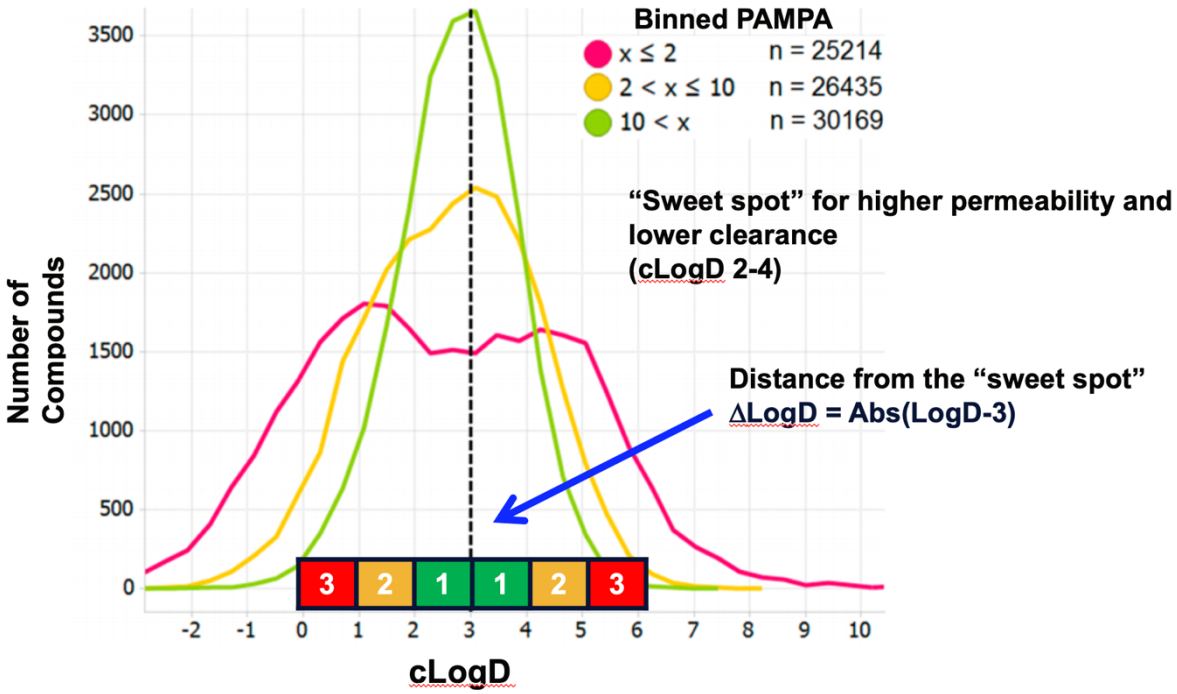
Whilst at times on a discovery programme drug space can seem a little crowded, Jean-Louis Reymond gave an insight into how vast chemical space really is. The GB-17 database contains 166.4 billion possible molecules up to 17 atoms of C, N, O, S and halogens following simple chemical stability and synthetic feasibility rules [10]. Using a set of MedChem rules and sampling this was filtered to produce GDBMedChem a compact collection of 10 million small molecules [11].

Visualisation of very large datasets is a significant issue and they have also been developing novel fingerprint and visualisation tools. Tmap is a very fast visualization library for large, high-dimensional data sets. A demonstration of Tmap displaying the 1.1 million ChEMBL dataset can be found here. http://tmap.gdb.tools/#ex-chembl.



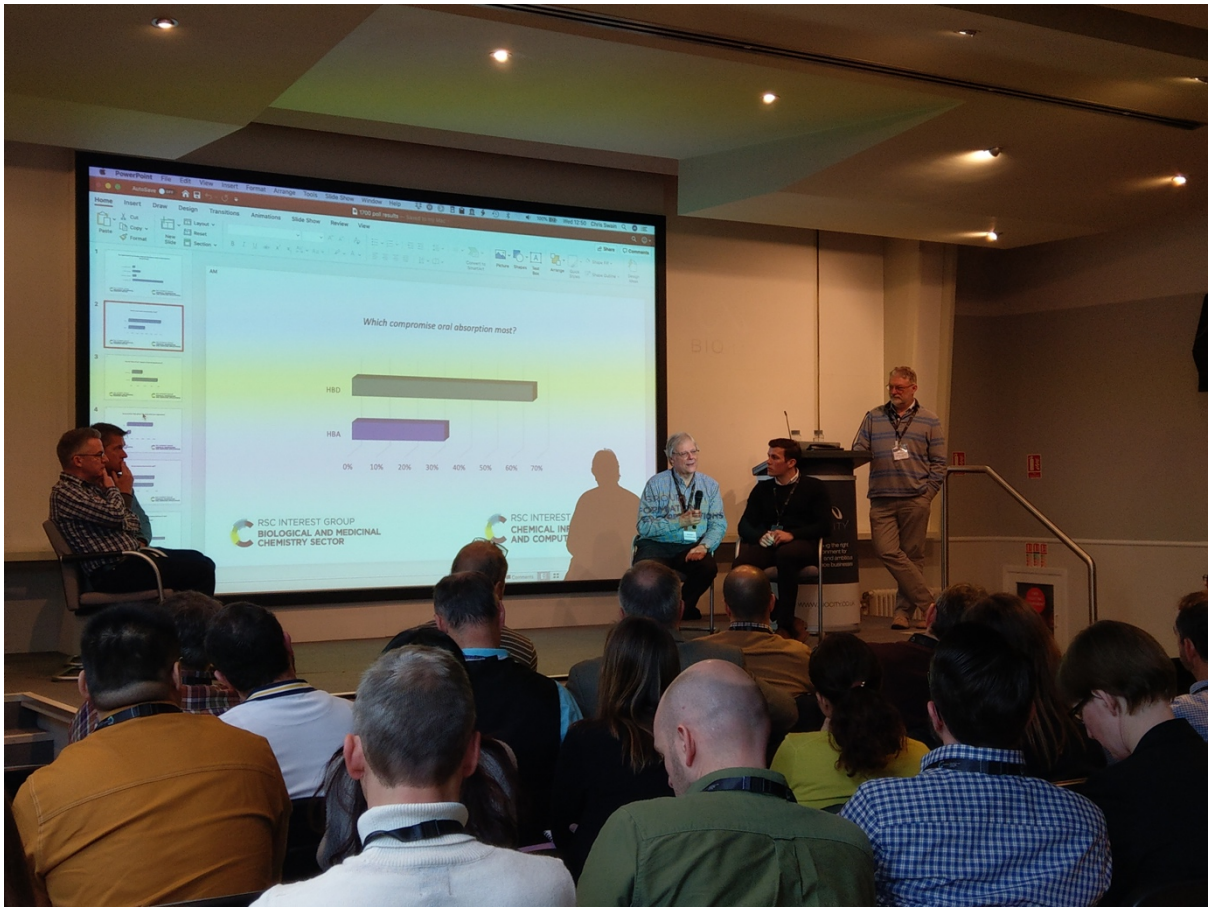
Most of the descriptors are quick and easy to calculate, however Jóhannes Reynisson (Keele Univ.) has chosen Density Functional Theory (DFT) to try and describe chemical space. Several properties were determined for known drugs, Dipole Moments, Polarisability, Ionisation potentials, Electron affinity. Higher Polarisability tended to be associated with poor oral availability, and lower dipole moment with higher bioavailability but there was considerable variation.

Phil Cox (AbbVie) finished the afternoon session by describing some of the lessons learned from AbbVie's work in the Beyond the Ro5 space (Bro5) [12]. Whilst many people have attempted to describe conventional drug space (Ro5), there are many difficult targets that require exploration of chemical space beyond the Ro5. In addition, whilst there is increasing amount of experimental data for compounds within the Ro5 space there is very little for Bro5. An analysis of the AbbVie DMPK database looking specifically at compounds Bro5 using a wide variety of calculated properties identified three properties that correlated Number of aromatic rings (NAR), Number of rotatable bonds (NRB), and the difference in cLogD from the mean cLogD of all compounds (LogD-3).



They proposed a simple multiparametric scoring function (AB-MPS) that correlated preclinical PK results with cLogD, number of rotatable bonds, and number of aromatic rings.

AB-MPS = Abs(LogD-3) + NAR + NRB, mnemonic for approximating odds of oral absorption for compounds bRo5.



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