Chemical, Target, and Bioactive Properties of Allosteric Modulation

Gerard J. P. van Westen*, Anna Gaulton, John P. Overington
Chemical Biology Group, European Molecular Biology Laboratory, European Bioinformatics Institute (EMBL-EBI), Hinxton, United Kingdom

Abstract

Allosteric modulators are ligands for proteins that exert their effects via a different binding site than the natural (orthosteric) ligand site and hence form a conceptually distinct class of ligands for a target of interest. Here, the physicochemical and structural features of a large set of allosteric and non-allosteric ligands from the ChEMBL database of bioactive molecules are analyzed. In general allosteric modulators are relatively smaller, more lipophilic and more rigid compounds, though large differences exist between different targets and target classes. Furthermore, there are differences in the distribution of targets that bind these allosteric modulators. Allosteric modulators are over-represented in membrane receptors, ligand-gated ion channels and nuclear receptor targets, but are underrepresented in enzymes (primarily proteases and kinases). Moreover, allosteric modulators tend to bind to their targets with a slightly lower potency (5.96 log units versus 6.66 log units, p<0.01). However, this lower absolute affinity is compensated by their lower molecular weight and more lipophilic nature, leading to similar binding efficiency and surface efficiency indices. Subsequently a series of classifier models are trained, initially target class independent models followed by finer-grained target (architecture/functional class) based models using the target hierarchy of the ChEMBL database. Applications of these insights include the selection of likely allosteric modulators from existing compound collections, the design of novel chemical libraries biased towards allosteric regulators and the selection of targets potentially likely to yield allosteric modulators on screening. All data sets used in the paper are available for download.

Introduction

Allosteric modulators

The generation of drug-like lead and candidate molecules against a specific molecular target remains a major challenge in drug discovery. We are now in a position to partially understand the factors behind this, and they fall into two basic themes – 1) the diversity and size of the set of compounds used in the initial screen, and 2) the physicochemical properties of the binding site of the target, which may contain obligate features that are incompatible to binding molecules with drug-like properties [1–7]. There are now a large number of ‘tantalizing targets’, those that have strong biological rationale (for example genetic validation), but are currently outside the reach of the development of novel small molecule therapies. One strategy to avoid the issues of factor 2) above is to consider the development of allosteric regulators, which may have better, or at least differentiated physicochemical properties or advantages in selectivity and so forth [8–11].

The concept of allosterism has received ample attention in literature, yet the term is used relatively loosely, the current work starts by defining the meaning of allosterism [8,12–17]. Allosteric modulators are ligands for a biological target that exert their effect on this target via a mechanism that is not located at the molecular site of action of those ligands that are the natural ligands or substrates for this protein. Hence the term ‘allosteric modulator’ covers a very broad spectrum of compounds and it depends on the context and function of the protein in question what effect allosteric modulators truly have. Thus, while some papers have previously been published classifying allosteric modulators as a separate class of ligands in general, here it is argued that the physicochemical properties of the molecules depend equally on the target in question [11,18].

For example if the target is a signaling protein (e.g. a G protein-coupled receptor (GPCR)) which naturally signals in response to ligand binding, an allosteric modulator can induce, inhibit, increase, or decrease this signal while still allowing the natural ligand to bind to the receptor (albeit with modified thermodynamic and kinetic parameters). In some cases the allosteric modulator can even prevent the natural ligand from binding through a conformational shift. Similarly, in the case of an enzyme, an allosteric modulator can increase, decrease, or block enzyme catalytic activity.

In the case of proteins with multiple functions and active sites, categorizing ligands as allosteric versus orthosteric can be problematic. For example, Figure 1 shows cyclin-dependant kinase 2 (CDK2) involved in cell cycle control and known to have multiple binding sites for which multiple inhibitor types exist [19]. Firstly, several inhibitors are known to inhibit the protein via the ATP binding site (which is commonly referred to as orthosteric inhibition, type I inhibition). Hence both ligands in competition with...
the ATP-binding site and proteins in competition with the substrate to be phosphorylated could be deemed orthosteric but differ significantly in their physicochemical properties. However, in literature the latter group is also classified as allosteric inhibitors. Moreover, one naturally occurring inhibitor of CDK2, cyclin-dependent kinase inhibitor 1B or cyclin-dependent kinase inhibitor p27, binds to the complex of CDK2 – cyclin A and protrudes into the ATP binding site [20].

Conversely, several small molecule classes have been identified that inhibit protein kinases in an allosteric manner. Type II inhibitors occupy the nucleotide-binding pocket and extend into the allosteric pocket, stabilizing the enzymatic inactive conformation (DFG out), whereas type III inhibitors bind and occupy an allosteric pocket. Additionally, there are type IV inhibitors that are covalent inhibitors targeting reactive proximal cysteine residues [19]. Finally, a fifth class (Type V) of inhibitors have also been discovered for CDK2. These are non-ATP competitive, but the binding pocket has been shown to differ from that of known type II and III inhibitors [21]. The inhibitors have been shown to bind near the C-helix, which is involved in the interaction of CDK2 with cyclin A and E [22,23]. Binding of these ligands also disrupts the protein – protein interaction (PPI) between CDK2 and cyclin, confirming the potential of allosteric modulators to disrupt PPIs.

Hence CDK2 is home to multiple binding sites to which multiple sets of ligands/substrates can bind in different ways. These ligands can be orthosteric (ATP-competitive), substrate competitive peptidomimetic molecules (non-competitive with regard to ATP, allosteric) or non ATP-competitive small molecules (allosteric), and can be further subdivided based on the mechanism of action. For these reasons, and because the approach here relies on retrieving allosteric papers, the term non-allosteric (rather than orthosteric) is used to describe other ligands binding to the same protein than those retrieved in the here described allosteric dataset. The definition of allosteric follows herein the target in question and general agreement in the literature; hence any observations are relative to this agreement in literature. There are a number of targets for which one can make similar distinctions with different forms of classification (e.g. in the kinase case one can define the ATP-competitive ligands as allosteric and only define the peptidomimetic ligands to be orthosteric). However, as the current results are derived from and based on medicinal chemistry literature it is chosen to follow this literature. Please see Case study 4 for further details on applying the here described methods to Kinase targets.

Allosteric modulators as drugs

As discussed above, the differences in binding site properties relative to the substrate/agonist/antagonist site are potentially attractive for operational drug discovery reasons. Allosteric modulators can hit targets with natural ligands that are outside classic oral drug-like space (e.g. class B GPCRs), or are difficult to hit with specificity with regard to paralogs (e.g. class C GPCRs), or...
can even be used to distort protein-protein interactions [24–26]. In all of these cases allosteric modulators can allow modulation of these targets by small molecules using well-established medicinal chemistry and drug delivery strategies.

Furthermore, allosteric modulators are interesting from a physiological viewpoint, as they provide a way to modulate natural regulation (amplify a naturally regulated response) rather than completely inhibit or continuously activate proteins. Orthosteric drugs activate or inhibit a protein in a dose dependent manner. Yet allosteric drugs can differ, while their concentration in the body is dose dependent, their effect can be dictated only by concentration but can also be dictated by concentration in combination with physiological signaling and feedback loops [15].

Finally, in GPCR signaling allosteric modulators have been shown to possess other advantages over orthosteric ligands due to functional selectivity displayed by these allosteric ligands. Functional selectivity is expected to lead to greater selectivity and safety of drugs targetingGPCRs [27].

However, there are also less favorable characteristics of allosteric modulators making them less suitable as drugs. By definition allosteric modulators inhibit non-competitively and often via a secondary binding pocket. Hence the shape and pharmacophoric properties of such a pocket are not necessarily as highly conserved across paralogs and orthologs, as a catalytic/substrate site would be. The former site will usually not be under the same selective evolutionary pressure for protein function as the latter [28]. In the case of viral inhibitors or any other systems where rapid genetic mutation and selection is possible (e.g. anti-fungals, anti-bacterials and anti-cancer therapeutic areas), the use of allosteric modulators might lead to easier onset of resistance by point mutations. This is empirically the case of the non-nucleoside reverse transcriptase inhibitors (NNRTI) used in the treatment of infections with the Human Immunodeficiency Virus (HIV). NNRTIs are well known for a quick onset of (cross) resistance [29]. Moreover, they are only effective on the HIV-1 subtype and not on the closely related HIV-2 subtype (61% identical when comparing HIV-1 strain M with HIV-2 strain A). In HIV-2 the allosteric pocket cannot be formed due to the presence of substitutions native to HIV-2, which lead to NNRTI resistance in HIV-1. Conversely, non-allosteric inhibitors are effective on both strains due to their similarity to the natural ligands [29,30].

Improvement of bioactivity models

Public resources like ChEMBL [31], Pubchem [32], BindingDB [33], and Drugbank [34] have transformed many parts of drug discovery. The availability of the data enables new research into signaling processes and the ligand – target bioactivity space [35–37]. For example, computational models can be developed using existing compound structure and activity data, and used to predict potential activities for other compounds. Hence this data opens the door for new applications like in silico side effect prediction, personalized medicine and rational design of polypharmacological drugs [38–40]. However the presence of multiple binding sites and binding modes potentially confuses and frustrates model development and validation in cases where multiple binding sites exist. Consequently the ability to distinguish between mode of action and systematic characterization of these compounds could potentially prove invaluable in drug discovery.

Aim of the work

In this work a top down analysis of allosteric modulators in the ChEMBL database was applied. Sets of ligands from papers in ChEMBL-14 were classified as being either allosteric, or non-allosteric (or presumed orthosteric) based on keywords, which were identified in both title and/or abstract. From the resulting papers the primary target was identified and then the compounds associated with this target were retrieved.

The resulting sets of ligands (allosteric and non-allosteric) are information dense (containing annotated target information, bioactivity, and the source documents). This information is subsequently exploited to study the allosteric concept over all bioactivities in ChEMBL, but also on a per target basis. Finally trends describing the chemistry, targets and bioactivity of compounds annotated to be allosteric are extracted.

Results/Discussion

Composition of data sets

Allostery has been reported in the ChEMBL database since the first indexed papers in 1980 (although the concept has been around in literature since the 1960’s) [12,13]. In total 987 unique documents were retrieved that together form the allosteric set (after manual curation for the case studies this number rises to 1,002). Likewise a non-allosteric set was retrieved, this set consisted of the documents that were not pulled in the first set and included the same restraints as applied to the allosteric set (see Methods). Finally a balanced non-allosteric set was derived from the full non-allosteric set to better perform unbiased classification. This balanced set was more similar in raw size and target distribution to the allosteric set (Table 1).

The allosteric records made up only a small fraction of the total records (around 3–4% of the total, Figure 2). However a trend was seen that the number of allosteric records have been increasing since the early 90’s with a peak in 2009–2010. Possibly this increase was caused by the recent focus on allosteric modulation of GPCRs [14,15,17,41,42]. While the total number of allosteric records in 2012 was lower, this was likely caused by the fact that ChEMBL-14 does not contain an entire years’ worth of 2012 publications. The full datasets are available for download on www. gipvanwesten.nl/allostery or ftp.ebi.ac.uk/pub/databases/chembl/

Allostery as are lists of all identified allosteric and non-allosteric activity_ids in ChEMBL-14.

Target distribution

The next obvious question was: what targets are amenable to allosteric modulation? This information could be useful in assessing the likelihood of finding an allosteric modulator for related targets, and can also be input to screening or assay strategies. Ideally this information leads to insights how theses targets differ from the targets preferentially interacting with non-allosteric modulators. Since allosteric modulators are sometimes a secondary approach when non-allosteric modulation is infeasible or impossible, the expectation would be that the target distribution is different. Recently, Li et al. published work where they studied targets that can be allosterically modulated [43]. Yet, their work was limited to targets with known crystal structures, and hence would suffer from a systematic bias to simpler globular proteins. Here this was taken a step further investigating all allosterically modulated literature targets that are retrieved from ChEMBL. The targets in ChEMBL are classified within a hierarchy, in which level 1 (L1) denotes the protein type (e.g. ‘Membrane Receptor’ or ‘Enzyme’), L2 further narrows the protein family (e.g. Class A GPCR known as ‘7TM1’) and so forth down to individual proteins (supporting Figure S1) [31].

Distinct differences were identified in the distribution of target classes when the total number of bioactivity measurements retrieved per target class was considered (L2 target distribution for both data sets, Figure 3). While the major target classes known
from medicinal chemistry literature were represented in both sets (e.g. class A GPCRs and Proteases) their distribution differed between sets, moreover there were major differences [4]. For instance class C GPCRs were enriched among the allosteric set, as were the Nuclear Receptors and the Ligand-Gated Ion Channels. For class C GPCRs it has traditionally been difficult to obtain selectivity using non-allosteric ligands as these ligands tend to be very small [41]. The tight structure-activity relationships observed,

|                      | Allosteric | Non-Allosteric (Balanced) | Non-Allosteric (Full) |
|----------------------|-----------|---------------------------|-----------------------|
| Years                | 1980–2012 | 1980–2012                 | 1980–2012             |
| Documents            | 1,002     | 8,315                     | 21,494                |
| Data points          | 18,281    | 18,035                    | 409,869               |
| Assays               | 2,111     | 9,938                     | 41,416                |
| Binding assay derived| 82%       | 81%                       | 86%                   |
| Functional assay derived| 17%     | 19%                       | 13%                   |
| Other                | 1%        | 0%                        | 1%                    |
| Targets              | 417       | 1,869                     | 2,935                 |
| L1 target classes    | 10        | 13                        | 13                    |
| L2 target classes    | 17        | 17                        | 22                    |
| Compounds            | 17,829    | 17,709                    | 384,288               |
| Small Molecules      | 97%       | 96%                       | 92%                   |
| Biologicals          | 3%        | 4%                        | 8%                    |
| Organic (Small Molecule) | 100%    | 99%                       | 100%                  |
| Inorganic (Small Molecule) | 0%     | 1%                        | 0%                    |
| Peptide (Biological) | 62%       | 46%                       | 42%                   |

Table 1. Data set composition.
centered around very ligand efficient recognition of the natural effector ligand do not allow much opportunity for variation in the receptor sequence and consequently in synthetic ligands binding this site (e.g. Metabotropic glutamate receptors, GABAB receptors, etc.). However it has previously been shown that selectivity can be obtained using allosteric modulation and this course of action has been pursued in the literature and was hence represented in the data set [17,41,44]. The overrepresentation of GPCRs was expected as it has previously been shown that GPCRs are targets typically readily accessible to allosteric modulation [16,42,45]. A similar plot has been created for the L1 target class, which can be found in the supporting information (supporting Figure S2).

Chemical structure properties

Similar to the target-based overview of allosteric versus non-allosteric compounds, the chemical properties of both classes of compounds were investigated to highlight differences (Figure 4A). The two most important observations were that historically identified allosteric modulators tend to fall within a much more narrower range of molecular weight (but are a subset of non-allosteric compounds rather than distinctly separated from non-allosteric compounds) and secondly that allosteric modulators adhered slightly better to Lipinski’s rule of 5 (75% versus 66%). Yet the important observation here was that the literature does not contain much information about allosteric modulators that are far from drug-like space. However, the relative scarcity of non-drug-like allosteric modulators does not mean that these are not possible (e.g. the peptidomimetic kinase inhibitors). A similar observation has been made by Wang et al. yet some examples of allosteric modulators outside drug-like space were retrieved here, contrary to their work [11]. One possible explanation for this lack of non-drug-like allosteric modulators could be based on the bioactivity statistics of allosteric modulators (see below).

The differences between allosteric modulators and non-allosteric modulators were further explored in Figure 4B where normalized activity was also included (based on a negative log value of IC50, EC50, Ki and Kd values). Overlap was observed in the high affinity locations shared by allosteric and non-allosteric ligands in a scatter plot showing compound fractional polar surface area and molecular solubility. Yet non-allosteric compounds also showed high affinity at fractional polar surface and molecular solubility values outside the values preferred by the allosteric compounds. From these observations it was concluded that the allosteric modulators in literature form a more restricted range subset (in the sense of physicochemical properties) from the overall set of compounds.

Combined, these results demonstrate that allosteric compounds are not distinct from non-allosteric compounds, however, given historical data, they appear to form a subset of the broad non-allosteric compounds (or medicinal chemistry derived compounds). The results also showed that allosteric compounds on average had a larger similarity between allosteric sets binding different target classes than between non-allosteric compounds binding different target classes (when considering physicochemical properties). The differences were further demonstrated using a case study where the chemical differences are relatively large between the two sets.

Case Study 1: Class B GPCRs

As touched upon in the introduction, the desirability of allosteric modulators for a certain target is not only governed by physiological or pharmaceutical demands. There are cases where orthosteric modulation is not feasible for the development of orally active small molecule drugs. Example cases are the class B GPCRs for which the natural effectors are polypeptide ligands of typical length ranging 30 to 40 residues [25,46]. There are many functionally and genetically validated links to pathology for this target class.

![L2 target class distribution of both the allosteric (A) and non-allosteric data (B) sets.](https://example.com/figure3.png)

Figure 3. L2 target class distribution of both the allosteric (A) and non-allosteric data (B) sets. The distribution of the target classes differed between the two sets; which confirmed that targets that are easy to hit via non-allosteric inhibitors are not necessarily easy to hit via an allosteric modulator and vice versa. Abbreviations: 7TM1 - Class A GPCRs, 7TM2 - Class B GPCRs, 7TM3 - Class C GPCRs, IP3 - Inositol trisphosphate receptors, KIR - Killer-cell Immunoglobulin-like Receptors, LGIC - Ligand Gated Ion Channels, RYR - Ryanodine Receptors, SUR - Sulfonylurea Receptors, TRP - Transient receptor potential channels, VGC - Voltage Gated Ion Channels.

doi:10.1371/journal.pcbi.1003559.g003
class, and a number of drugs are available (some examples are iv/sc dosed - Calcitonin (Miacalcin), Exendin-4 (Exenatide), and PTH (Forteo)) [25,47–49]. This target class was represented approximately equal in both the allosteric and non-allosteric data set (0.3% of the allosteric and 0.6% of the non-allosteric papers). While no large differences were apparent in the target distribution, the physicochemical properties of compounds annotated as allosteric modulators differed from those annotated as non-allosteric modulators. Figure 5 summarizes some of the findings for the class B GPCRs as retrieved from the data set. A figure with all 68 descriptors used (supporting Table S1) is also available (supporting Figure S3). In addition all data is available in tab delimited text format on www.gjpvanwesten.nl/allosterism or ftp.ebi.ac.uk/pub/databases/chembl/Allosterism. Here a limited figure is displayed for reasons of clarity.

Differences in physicochemical properties were found for allosteric and non-allosteric class B ligands (Figure 5). The non-allosteric (peptide like) ligands were very large (Mwt range 334 Da to 3591 Da for 95% of the data) whereas those ligands annotated to be allosteric modulators were ‘classical’ small molecules (Mwt between 305 Da and 569 Da for 95% of the data). Hence, differences were observed in properties related to size like: the number of chains or the number of hydrogen bond acceptors. However, when corrected for the size of the ligands, the differences were less distinct (e.g. carbon fraction of the total atoms). Interestingly the allosteric ligands were more rigid as indicated by a higher sp2 hybridized carbon fraction, lower sp3 hybridized carbon fraction, higher aromatic bonds fraction, and higher rigidity index (see methods for a further explanation of the rigidity index). Allosteric ligands tended to pass the Lipinski rule of five (60%) and were more drug-like, whereas non-allosteric ligands were less prone to pass Lipinski’s rule (30%) and were not drug-like (Figure 5). Finally, the average formal charge for allosteric ligands was slightly negative and slightly positive for non-allosteric ligands. Similar charts have been created for all other significantly populated target classes (L2) and can be found on www.gjpvanwesten.nl/allosterism or ftp.ebi.ac.uk/pub/databases/chembl/Allosterism.

Secondary to physicochemical properties, substructures that are overrepresented in either the allosteric ligands or the non-allosteric ligands for a target class are of interest. Hence for each target class all present substructures (using circular fingerprints FCFP_6) were retrieved and their frequency in the allosteric and non-allosteric sets were compared against the background of the combined (full) set. Substructures were then sorted based on the enrichment score (supporting Table S2, Table S3, and Table S4). The results were in correspondence with what would be expected considering the natural ligands for these receptors and the observations from Figures 4 and 5. Substructures ranking high based on their allosteric score were quite specific, and tended to be aromatic. Conversely, substructures ranking very low based on their allosteric preference were small, frequently occurring and mainly introducing polarity. Interestingly, substructures scoring high based on their non-allosteric score included protein backbone like structures. The full set for all L2 target classes is available as a download from www.gjpvanwesten.nl/allosterism or ftp.ebi.ac.uk/pub/databases/chembl/Allosterism.

Bioactivity of allosteric modulators

Protein targets and chemical properties of ligands in the allosteric set and the non-allosteric set were the point of focus in the above text. Now the differences between the bioactivity of allosteric compounds and the bioactivity of non-allosteric compounds are summarized. Considered were: potency (affinity), the number of targets that compounds from both groups have been tested on, the number of targets compounds from both groups were active on, the Ligand Efficiency (LE) [50], and a number of other
efficiency indices (Binding Efficiency Index (BEI), Surface Efficiency Index (SEI), Normalized Surface Efficiency Index (NSEI), etc. [51,52](Table 2)

The median potency was lower for allosteric modulators than for non-allosteric modulators (5.96 log units versus 6.66 log units, p < 0.01). Moreover, a lower fraction of the compounds was considered ‘active’ (33% versus 47%), with activity being defined operationally as potency better than micromolar (6 log units) or annotated ‘active’ in the source data. Likewise a higher fraction was inactive (39% versus 29%, less than 6 log units or annotated ‘inactive’ in the source data). Allosteric modulation is a process that cannot be explained by only ligand affinity (the dynamics are much more complicated and the reader is referred to a number of reviews) [53,54], yet the current findings with regard to affinity are discussed here given the importance of this measurement in drug discovery.

Several possible explanations for the observed differences can be considered. Firstly, it is known that metabolites can be allosteric regulators and these metabolites can be present locally at very high concentrations and can hence exert their effect with a relatively low potency. Table 2 could implicate that the data set reflects the presence of metabolites in our dataset, annotated as allosteric ligands. High concentration metabolites would not need micromolar affinity when they are present at a millimolar concentration locally [8,55,56]. Secondly, another explanation can be that the optimization of high affinity allosteric binders is more challenging given the more constrained chemical characteristics that allosteric modulators display compared to non-allosteric modulators. However, there are two more likely but also more complex potential explanations for the observed lower affinity as will be described below.

Rationalizing the observed lower affinity of allosteric modulators

A third explanation for the observed lower affinity could be derived from observations in the field of GPCRs. The current work is not the first to observe a lower affinity for allosteric modulators compared to non-allosteric interactions, in particular in the field of GPCRs this has been observed before [54]. While GPCRs are a complex modeling system given the baseline presence of both an orthosteric (natural ligand) and allosteric (G protein) binding site in all GPCRs, there are some observations that can perhaps be translated to a more general view of allosterism. It has been shown that allosteric interactions have a direct effect on the affinity of non-allosteric ligands (orthosteric in GPCRs) [54]. Given that affinity is defined as the ratio of ligand association to ligand disassociation rates, allosteric modulators directly affect the non-allosteric (dis)association rate. However, the allosteric interaction between two sites has been shown to be reciprocal [54], hence the affinity of allosteric modulators is influenced by the affinity of non-allosteric modulators. As such the observation of the lower affinity of allosteric modulators might be a product of the dominant usage of radio-ligand binding assays (as follows). Typically radio-ligand binding assays are set up using a well-known ligand, a radioactive molecule is synthesized based on this ligand and the binding of uncharacterized
molecules is explored through their effect on the radio-ligand. Given that the radio-ligand is usually a well-known ligand, it is often a ligand with a reasonably high affinity. Hence this high affinity effect might influence the observed affinity of allosteric ligands due to the reciprocal nature between the binding site of an allosteric ligand and a non-allosteric ligand. When comparing competitive inhibition between two non-allosteric ligands (radio ligand and unknown molecule) this effect will likely not be present. While this explanation is funded on observations from the field of GPCRs, it should be noted that in this field allosteric modulation has arguable been the most intensely explored.

Another observation from the field of GPCRs is that ligand efficacy does not necessarily correlate to ligand affinity. There is documented evidence in literature wherein the ligand with the best affinity does not display the best efficacy [57,58]. It has been hypothesized that this discrepancy can partially be explained through the concept of binding kinetics. For a number of GPCRs it has been found that efficacy is better explained when receptor residence time or dissociation rate is considered (the most efficacious ligands are shown to be the ligands with longer residence time) than when only affinity is considered [57–59]. In the case of allosteric modulators a similar principle might apply. Indeed, cases in which allosteric modulators modify binding kinetics of non-allosteric ligands have been described in literature [60,61]. Given that we observe here that allosteric modulators tend to be relatively small and lipophilic molecules one can expect de-solvation to play a major role in binding kinetics. Hence these molecules might display a baseline longer residence time than non-allosteric molecules due to their physicochemical properties. However, further research and experimental evidence is required to confirm or reject this hypothesis.

### Implications for assays focused on allosteric modulation

While any classification into ‘active’ or ‘inactive’ is based on a cut-off, the observations here regarding affinity illustrate a larger issue. In screening efforts cut-offs are important to retrieve interesting ligands. If the median potency of allosteric modulators is lower than that of non-allosteric modulators (corroborated by the tendency of allosteric ligands to be smaller, to be more lipophilic, and to possess less hydrogen bonding potential) this could very well lead to possible allosteric modulators being missed in screening efforts. The general threshold for activity in primary screening is 10 μM to find compounds that are shown to have a median activity of 6.66 log units in ChEMBL. Hence, the implication would be that any screening effort for allosteric modulators (median activity of 5.96 log units) would need to be more sensitive or at least have the definition of ‘active’ adapted to conform to our observations. Moreover, given the reciprocal nature of the effects that allosteric and non-allosteric sites have on each other, it would be recommended to not use a single radio-ligand if one is aiming to find new allosteric modulators. A better choice is to use a spectrum of assays with different radio labeled ligands as has also been suggested by May et al. [54].

That said, allosteric compounds were found to have similar but slightly higher median binding efficiency indices (LE, BEI, SEI, NSEI, NBEI) instead of the difference being likely caused by the fact that allosteric modulators tend to be smaller than non-allosteric modulators. This potentially indicates on average smaller, less polar binding sites for allosteric versus non-allosteric classes [43]. Moreover, we observed that allosteric modulators tend to have been annotated to a lower number of targets (2 versus 3) but this difference is marginal. Additionally, the median number of targets a compound is active on is shown to be 1 (average 1.43) for the non-allosteric set, in line with the findings of Hu and Bajorath [62], but the values are median 0 (average 1.40) for the allosteric set.

In conclusion, allosteric modulators were found to be able to modulate targets with low affinity but high efficiency. In addition, the data did not show allosteric modulators to be inherently promiscuous binders – at least as inferable from the distribution of assays reported in ChEMBL –, rather there was a trend for

### Table 2. Bioactivity measurements for allosteric and non-allosteric compounds.

|                      | Allosteric Median (MAD) | Non-Allosteric Median (MAD) |
|----------------------|-------------------------|-----------------------------|
| Activity             | 5.96 (±1.02)            | 6.66 (±1.17)                |
| LE                   | 0.319 (±0.0723)         | 0.310 (±0.0689)             |
| BEI                  | 16.3 (±3.69)            | 15.9 (±3.55)                |
| SEI                  | 11.1 (±4.12)            | 10.3 (±3.82)                |
| NBEI                 | 0.233 (±0.0528)         | 0.226 (±0.0503)             |
| NSIE                 | 1.16 (±0.370)           | 1.08 (±0.348)               |
| nBEI                 | 7.34 (±1.06)            | 8.12 (±1.19)                |
| mBEI                 | 8.49 (±1.06)            | 9.27 (±1.19)                |

A threshold of 6 log units was used to classify compounds as ‘active’. Abbreviations: MAD – Median Average Deviation, LE – Ligand Efficiency (kcal/mol per non-hydrogen atom), NBEI – Normalized Binding Efficiency Index (non-hydrogen atoms), BEI – Binding Efficiency Index (molecular weight), SEI – Surface Efficiency Index (polar surface area/100), NSEI – Normalized Surface Efficiency Index (polar atoms), nBEI – Normalized Binding Efficiency Index taking the log after calculation of the ratio (non-hydrogen atoms), mBEI – Normalized Binding Efficiency Index taking the log after calculation of the ratio (molecular weight). See Abad-Zapatero et al. [32].

doi:10.1371/journal.pcbi.1003559.t002
allosteric compounds to be less promiscuous than non-allosteric modulators, which is also seen in previous work [43]. While the potential of the current data set is demonstrated by comparing the allosteric and non-allosteric set, this analysis is by no means exhaustive. Similar analyses can be performed comparing different allosteric sets or for instance comparing class C GPCR ligands from the allosteric set with the class A GPCRs of the non-allosteric set (comparing two different sets of trans-membrane domain binding ligands). Moreover, it should be noted that further research is required to determine if the lower binding affinity observed results from database bias or if this is an intrinsic property of allosteric modulators (and if so, what the cause is of this observation).

**Allosteric classification models**

Above it was shown that there are chemical differences between allosteric ligands for a certain target class and non-allosteric ligands for that same target class. In some cases these differences were large (as in the case of class B GPCRs) whereas in other cases the differences appeared to be smaller (as in the case of class A GPCRs). These chemical distinctions were used to train a classification model that would be able to predict if a compound would likely be an allosteric modulator or a non-allosteric modulator for a given target based on the physicochemical properties. These models were created on the balanced set to avoid a large bias in classifier predictions (Table 1). Non-balanced models have also been trained and data is available in the supplementary information.

The use of (circular) fingerprints in the full (non-target specific) models was sidestepped for several reasons; firstly these models should have a large applicability domain and should hence not be limited to certain chemical motifs. Secondly, (chemical) sampling bias of specific historical target classes was to be avoided. Thirdly, the large chemical diversity would probably make those features that are predictive very generic (as shown in the class B GPCR case study for substructures negatively associated with allosteric modulators, Supporting Table S2, S3, S4). Finally the improvement of circular fingerprints to the models was marginal (on average 5% as calculated by the average of the used parameters, Supporting Table S5). Hence circular fingerprints were only used in more congeneric chemical sets (e.g. target specific) [63]. Models were judged by recall of allosteric modulators (Sensitivity (sens)); recall of non-allosteric modulators (Specificity (spec)); precision for allosteric modulators (Positive predictive value (PPV)); precision for non-allosteric modulators (Negative predictive value (NPV)); and Matthews correlation coefficient (MCC). These were all 0 for a non-predictive/random model and 1 for an ideal model with the MCC also potentially being -1 for an ideal inverse model (see Methods for further details).

Table 3 shows a selection of the results for allosteric classification models (each trained on 70% of the data and externally validated on the remaining 30%). For the full table see supporting Table S6, here we limited ourselves to a single page for reasons of clarity. Different models on data sets grouped by class L0 (protein binding compounds), L1 (first level classification), and L2 (second level classification) have been trained. Figure 6 shows the out-of-bag ROC curve and external validation for the L0 model. For all groups models were able to classify a compound as allosteric modulator or non-allosteric modulator of a given target class with good accuracy, yet model performance improved when sets became more specific (limited to a target class). These models provide a useful tool for the elucidation of the mechanism of action for compounds identified in primary HTS screening efforts.

Second to being able to predict if a compound will or will not be an allosteric modulator, it is also of interest to find out what properties are important to make this distinction. Given in Table 3 are the three most important properties that were correlated with the ‘allosteric’ class and the three most important properties that were correlated with the ‘non-allosteric’ class for each classification model. These properties allow the further investigation into what differentiates allosteric from non-allosteric compounds. While in most cases allosteric modulators were more lipophilic and non-allosteric compounds were associated with a higher polar surface area this was not always the case. Examples were the Transient Receptor Potential Channels (TRP) and Voltage Gated Ion Channels (VGC) target classes (L2 target class, ion channels), part of the Ion Channel (L1 target class). Here allosteric ligands had a larger polar surface area (TRP) or larger polar solvent accessible surface area (SASA) (VGC). Conversely non-allosteric ligands were more rigid (TRP). No explanation for this observation is currently available but possibly, in the case of these two ion channels, the uncompetitive binders could bind near the ion channel itself and hence resemble these ions that are transported by these proteins rather than resembling the natural regulators (which is Voltage in the case of VGC and can be diverse in the case of TRP). Note that this observation was absent for the Ligand Gated Ion Channels (LGIC) where the allosteric modulators seem to correspond more to what we observe in other protein classes (double bonds are favorable and solubility/positive atom fraction are not favorable). For the full table containing the results of all classification models trained on all targets in levels 0–2 (including class ‘undefined’ models) see supporting Table S6. In the final section the potential of the data set is demonstrated using three further different case studies.

**Case Study 2: HIV reverse transcriptase**

To illustrate possible applications of the data set, the classification models were applied to a number of previously studied targets for which a range of allosteric inhibitors has been published. The first of these targets is the viral enzyme HIV-1 reverse transcriptase (HIV-RT), for which substantial SAR data and several approved drugs are well established [64,65]. A relevant drug target in the treatment of HIV, this target will fall into ‘Enzyme’ L1 target class and is not further defined on lower target class levels due to the sparseness of other related proteins in version 14 of ChEMBL. Importantly, both allosteric and non-allosteric drugs have been successfully developed as therapeutics, and many co-crystal structures reported clarifying the binding sites of various compound classes, making this an ideal target case. Furthermore rational design and random screening have been used to extensively study the protein.

Before training models the molecules were clustered based on FCFP_6 fingerprints. As can be expected there were some mis-classifications in the dataset. Known allosteric compounds were in the non-allosteric training set (sharing scaffolds with known allosteric inhibitors). Moreover, a number of compounds in the allosteric training set were noted to be non-allosteric compounds (nucleotide like structures) and vice versa. Capturing this unannotated, or tacit knowledge within a field is challenging, and highlights some issues with data-mining the literature where ad hoc vocabularies and conventions are used; however, it also highlights the opportunity and added value for further curation. The clusters containing these compounds were reclassified based on the information in the original publications and subsequently a model was trained (Table 4). The model performed well with a sensitivity of 0.89, specificity of 0.88, PPV of 0.92, NPV of 0.84 and MCC of 0.76 and was hence interpreted (Figure 7).

The HIV-RT allosterism model showed the three most important descriptors for non-allosteric compounds to be fraction of Oxygen atoms as a part of all atoms (for instance the presence of
### Table 3. Examples of allosteric models for balanced data sets of L0, L1, and L2 groups.

| Target Level | Class       | Allosteric | Non-allosteric | Sens |Spec | PPV | NPV | Allosteric property 1 | Allosteric property 2 | Allosteric property 3 | Non-allosteric Property 1 | Non-allosteric Property 2 | Non-allosteric Property 3 |
|--------------|-------------|------------|----------------|------|-----|-----|-----|-----------------------|-----------------------|-----------------------|--------------------------|--------------------------|--------------------------|
| 0            | n/a         | 18,281     | 18,035         | 0.83 | 0.82 | 0.82 | 0.83 | Doublebonds Frac     | Carbon Frac            | LogP                  | drugLikeness             | PSA Frac                | Stereatom Frac           |
| 1            | Enzyme      | 9,531      | 8,425          | 0.84 | 0.81 | 0.83 | 0.82 | Doublebonds Frac     | Aromatic Bonds Frac   | Sulphur Frac           | drugLikeness             | Molecular PSA            | Num Chains              |
| 1            | Membrane Receptor | 1,974 | 1,966         | 0.86 | 0.88 | 0.88 | 0.86 | LogD                  | LogP                  | Sulphur Frac           | Solubility              | H Donor Frac             | Heteroatom Frac          |
| 2            | 7TM1        | 2,130      | 2,081          | 0.89 | 0.89 | 0.89 | 0.89 | Rigidity Index       | Carbon Frac            | Polar SASA Frac        | Num sp3 Carbons         | Hydrogen Frac            | Sp3 Carbon Frac          |
| 2            | 7TM2        | 119        | 107            | 1.00 | 0.88 | 0.90 | 1.00 | sp2 Carbon Frac     | Carbon Frac            | Rigidity Index         | Num Chains              | SP3 Carbon Frac          | Singlebonds Frac         |
| 2            | 7TM3        | 2,192      | 2,196          | 0.91 | 0.88 | 0.89 | 0.91 | Num Chain Assemblies | Carbon Frac            | LogP                  | Positive Atom Frac      | Sp3 Carbon Frac          | Cmp Zwitterion           |
| 2            | Kinase      | 1,461      | 1,419          | 0.90 | 0.88 | 0.89 | 0.90 | Oxygen Frac          | Molecular SASA         | Num Terminal Rotomers  | Nitrogen Frac            | Ringbonds Frac           | Num Rings               |
| 2            | LGIC        | 1,803      | 1,791          | 0.86 | 0.89 | 0.87 | 0.86 | Sulphur Frac         | Num Chains             | Doublebonds Frac       | Solubility              | H acceptor Frac          | Positive Atom Frac       |
| 2            | TRP         | 106        | 109            | 0.96 | 0.92 | 0.92 | 0.96 | Ringbonds Frac      | Num Halogens           | Molecular Surface Area | Molecular PSA            | Num Terminal Rotomers    | Rigidity Index           |
| 2            | VGC         | 43         | 39             | 1.00 | 1.00 | 1.00 | 1.00 | Molecular PSA        | Rotatable Bonds Frac  | Num Terminal Rotomers  | Ringbonds Frac           | Hydrogen Frac            | Molecular Volume         |

Abbreviations: 7TM1 – Class A GPCRs, 7TM2 – Class B GPCRs, 7TM3 – Class C GPCRs, LGIC – Ligand Gated Ion Channels, TRP – Transient receptor potential channels, VGC – Voltage Gated Ion Channels, Frac – Fraction, Cmp – compound, H Acceptors – Hydrogen Bond Acceptors, H Donors – Hydrogen Bond Donors, LogD – distribution coefficient, LogP – partition coefficient, PSA – Polar Surface Area, SASA – Solvent Accessible Surface Area, sp2 – SP2 Hybridized Carbons, sp3 – SP3 Hybridized Carbons, Num – Number of, n/a – Not Available.
a ribose moiety or a number of phospho groups contributes to this descriptor), a larger polar surface area and a larger fraction of atoms that are H-bond acceptors. Conversely, the following parameters were found to be predictive for allosteric ligands: a larger fraction of the bonds should be aromatic, the fraction of bonds that are ring-bonds should be higher and the distribution coefficient (LogD) should be higher (for a top 20 list see supporting Table S7). These results demonstrate that the here-published data set is a suitable starting point to create a model that can differentiate between likely non-allosteric and likely allosteric ligands for a specific target. However, after further data set curation this approach can lead to a well performing model that can reliably differentiate between these classes. This approach to developing a predictive method for allosterism is however not limited to enzymes as is shown in the following examples.

Case Study 3: Adenosine receptors

Like HIV-RT, the class A GPCR adenosine receptors form a highly validated and important drug target, where both agonists and antagonists have a therapeutic potential. Moreover, there is now structural data for this GPCR target. Adenosine receptors are relevant targets in the treatment of diabetes and Parkinson’s disease [66]. Allosteric modulation of the adenosine receptors has anticipated advantages over orthosteric modulation as it is expected to increase tissue specific selectivity and enable modulation of receptors present in the brain [66]. Moreover, class A GPCRs make up a large fraction of the targets present in ChEMBL. This is due to their high relative tractability, the historical research effort on this class, the large size (ca. 300 family members in the human genome), and linkage to many important diseases [4]. However, unlike HIV-RT no allosteric modulators of adenosine receptors have yet been launched as drugs. One compound, T-62, was under evaluation for the treatment of chronic pain but crashed out in phase 2 trials [67]. Moreover, there is a preclinical body of work that demonstrates allosteric modulation for these drug targets and hence they were chosen to be included here as a case study. Different from the HIV-RT case study is that here a group of closely related proteins is used rather than a single target. Hence it is shown that the current data set can also be used to capture properties that distinguish allosteric modulators for a family of targets.

Again some manual curation was needed before moving to model training. The main finding was the paper by Narlawar et al. [68]. This paper describes bitopic ligands that possess both allosteric and non-allosteric domains. The compounds were marked as allosteric due to the keywords noted in the abstract, yet the large non-allosteric part of the ligands (including a ribose moiety) deteriorates model performance. Similarly a number of ligands described by Jacobson et al. were included in the allosteric set as the abstract mentions that only some compounds appeared to bind at an allosteric site, yet the majority of the 78 compounds were non-allosteric, hence these were also cleaned [69].

The adenosine receptor allosteric modulator model performed well (sens 0.94; spec 0.97, PPV 0.66; NPV 1.00; and MCC 0.77; Figure 7), although the lower PPV lead us to believe further curation might improve model performance. The model was then interpreted. Allosteric ligands had a higher fraction of aromatic bonds, a higher LogD, and a higher average bond length compared to non-allosteric ligands. Whereas non-allosteric ligands had a higher heteroatom fraction and a larger polar surface area compared to allosteric ligands. Yet there was an interesting distinction with the HIV-RT models. The structures of known adenosine ligands (both allosteric and non-allosteric) are much more conserved than those of HIV-RT ligands. Hence structural features (in this case FCFP_6 substructures) were much more important in model creation compared to generic physicochemical properties (for example a xanthine scaffold was found to be correlated with non-allosteric modulators, supporting Table S8).

Three substructures were shown to have high importance values in model creation (meaning that model quality significantly decreased by leaving them out of the descriptor set).

Case Study 4: Kinase modulators, protein Kinase-B

A fourth and final case study presented in this paper is protein Kinase-B (PKB)/Akt 1. This enzyme target is relevant in oncology as it plays an important role in cellular survival pathways by

Figure 6. (A) Receiver Operator Characteristics (ROC) curve for out-of-bag validation of the allosteric classifier trained on 70% of the allosteric and balanced orthosteric set demonstrated good performance. (B) External validation on the remaining 30% of the data set confirmed good predictive performance. doi:10.1371/journal.pcbi.1003559.g006
Table 4. Overview of allosteric models used in the case studies.

| Target          | Type                  | MCC (Sensitivity) | Non-Allosteric Recall (Specificity) | Allosteric Biologicals Precision | Non-Allosteric Precision (PPV) | Allosteric Biologicals Recall | Non-Allosteric Recall (PPV) |
|-----------------|-----------------------|-------------------|-------------------------------------|----------------------------------|--------------------------------|------------------------------|-------------------------------|
| HIV Reverse     | Single target         | 0.76              | 0.94                                | 0.97                             | 0.99                           | 1.00                         | n/a                           |
| Transcriptase   | Target group          | 0.77              | 0.94                                | 0.97                             | 0.99                           | 1.00                         | n/a                           |
| Protein Kinase B| Multiple allosteric classes | 0.86             | 0.96                                | 0.94                             | 0.94                           | 0.99                         | 1.00                          |

In the case of Protein Kinase B a three-class model was created (natural ligand mimicking peptides formed the third class). For class errors, sensitivity is recall of allosteric small molecules, specificity is recall of non-allosteric small molecules, and negative predictive value (NPV) is precision for non-allosteric small molecules. Note that the value for the non-MCC parameters in the three-class model have been scaled to the same range as the binary classification models to allow direct comparison. Abbreviations: MCC – Matthews Correlation Coefficient, HIV – Human Immunodeficiency Virus.

Prospective use of allosteric classifiers

In the case studies the potential of the data set identified and provided in this paper is demonstrated. The dataset is shown to be a solid starting point for allosteric focused drug discovery towards existing targets or towards new targets. With modest further curation highly predictive models could be obtained. While it is outside the scope of this paper to provide a case study on all potentially interesting protein targets, possible other examples included in the set are (but not limited to): Kinesin EG5 [72–74], inhibiting apoptotic processes [70,71]. PKB differs from the previous targets as two different classes of allosteric modulators have appeared in the literature. As touched upon in the introduction, allosteric modulators of kinases can be small molecules that act for instance by shifting the balance of protein dynamics (e.g. locking a protein in an inactive conformation). However in the case of kinases where orthosteric modulators are defined as ATP-competitive, allosteric modulators can also be compounds that resemble the substrate of the kinase and hence be peptides (protein like compounds). In the current case study the allosteric modulators hence make up two major classes, one of which are large peptide like compounds. As such Protein Kinase B is an interesting target that forms the inverse of the class B GPCRs mentioned above. The non-allosteric modulators in this case were all ATP-competitive and it was hypothesized that this class forms a group that is more similar chemically than the allosteric modulators. Given the clear distinction between allosteric modulators that are peptidomimetic and small molecule allosteric modulators, the chosen course of action was to train the model using a three-class model rather than a binary classification model. The model had good predictivity (sens 0.96; spec 0.94; PPV 0.71; NPV 0.99; and MCC 0.86; Figure 7); the added third class, ‘allosteric biological’, was predicted very well with recall 1.00 and predictive value 1.00.

As expected, properties mostly related to size (Molecular polar surface area, volume) were correlated with the biological allosteric modulators as is the ChEMBL calculated molecular class ‘biological’. The physicochemical properties mostly correlated with small molecule allosteric modulators were number of chain assemblies, ringbond fraction, carbon fraction, and number of sp2 hybridized carbons. Additionally the ChEMBL calculated molecular class ‘small molecule’ was correlated to small molecule allosteric modulators. Interestingly, properties Lipinski pass, aromatic bonds frac, ringbonds frac, and LogD were also correlating with non-allosteric modulators (contrary to the trends observed in other targets). This is likely due to the fact that ‘small molecule allosteric modulators’ and ‘small molecule ATP competitive modulators’ more closely resemble each other than they do the ‘biological allosteric modulators’ in terms of physicochemical properties. Moreover the non-allosteric/ATP-competitive set contained a number of drugs, which are highly optimized structures. Yet, LogD, and ringbonds fraction correlated to both the allosteric and non-allosteric small molecule classes. Conversely, negative atom fraction and number of hydrogen bond acceptors were correlated with only non-allosteric compounds (likely due to the need for ATP-competitive compounds to also resemble parts of ATP), but this effect was less pronounced. Also in this case study (similar to HIV RT) sub-structural features were observed to be very important. Moreover, in the biological allosteric modulators class protein/peptide backbone fragments were appearing as important in combination with charged arginine side chains. Inversely, in the case of small molecule allosteric modulators the important substructures mostly contain aromatic rings. For a longer list see supporting Table S9.
Alcohol dehydrogenases (e.g. Isocitrate dehydrogenase 1 and 2 [ICDH]) [75], and class C GPCRs [26].

The models obtained here trained on the full allosteric modulator set should have a broad domain of applicability due to their generic nature (physicochemical properties were used as descriptors). Hence it is expected that these models are not limited to certain known chemical motifs as would be the case when using circular fingerprints. While also outside the scope of the current paper, the authors would very much welcome a prospective validation of the models. It should be noted that these models are solely classifying between 'a likely allosteric interaction' and 'a likely non-allosteric interaction'. Hence the models cannot be used to predict the affinity of ligands on certain targets, but are able to predict the likely type of interaction for a given interaction. As such these models should ideally be combined with dedicated bioactivity models that can predict the affinity of molecules on a certain target and not replace them. Hence the allosteric classifiers can be used as a secondary filter when selecting compounds from a chemical vendor to be tested experimentally.

The authors feel that other potential applications could be the following: Firstly, creation of allosteric focused libraries based on known chemical properties of allosteric modulators, these libraries can be further sub divided on target type (e.g. Class A GPCR or Protein Kinase). Secondly, determination of interaction type of hits retrieved from HTS screening (allosteric or non-allosteric).

The authors are very open for potential collaborative projects to experimentally validate the approach as described here. Hence the authors would urge readers to contact them when they are interested in a specific set of allosteric modulators.

Conclusions
As stated in the introduction, the term allosteric modulator is a very broad definition directly depending on the target (class) in question. Despite the presence of peptidic ligands and very diverse chemistry, there are some general conclusions that can be drawn from the current work.

Allosteric modulators tend to be more rigid and lipophilic structures compared to the background set. This is in line with their mode of action via binding in distinct structural locations of proteins rather than catalytic or agonist sites. Yet the magnitude of these changes in physicochemical properties depends on the target in question and the non-allosteric ligands. Moreover, it is observed that allosteric modulators are constrained to a narrower structure activity window than are non-allosteric modulators. When the physicochemical properties of allosteric modulators are compared to all ligands for a target, the allosteric modulators are often a subset of the non-allosteric ligands.

Secondly, it is observed that allosteric modulators are interesting drugs for several reasons. They tend to adhere better to Lipinski’s rule of 5, making them good candidates for oral formulation. This could indicate that, if allosteric hits are identified for a target, allosteric ligands are more developable then non-allosteric ligands. Moreover, a trend is observed that allosteric modulators are less promiscuous than non-allosteric modulators.

Thirdly, the absolute potency for allosteric modulators is observed to be lower, while their binding efficiency and surface ligand efficiency is similar. Some potential causes are discussed here, but before a qualitative statement can be made about this observation further research is required. However this observation does call for the adaptation of screening assays to pick up the lower affinity compounds.

In conclusion, the differences between non-allosteric and allosteric modulators for a given target are usually such that it is not straightforward to turn a non-allosteric compound into an allosteric compound or vice versa. Yet it is these chemical differences that allow the creation of classification models that can distinguish between allosteric and non-allosteric modulators. These models are shown to perform better if the target definition is more concise, yet even without these constraints already predictive models were constructed. Hence non-allosteric and allosteric inhibition of a single target can be considered different target classes overall. The work performed here should lead to improvement of bioactivity models by providing tools to incorporate binding mode as a descriptor for compounds and hence reducing the noise present in a data set.

While the authors have demonstrated in the current paper how the dataset can be used as a starting point for allosteric drug design, full manual curation of the dataset is at the moment infeasible. Hence the authors encourage everybody who encounters an error or misclassification in this data set to contact them so that curation can take place via crowdsourcing and the quality of this data in ChEMBL can increase.

Figure 7. ROC curves for out-of-bag validation of the allosteric classifier models trained in case studies 2–4. (A) ROC curve for the HIV-RT classifier. (B) ROC curve for the adenosine receptors classifier. (C) ROC curve for the Protein Kinase B classifier (note that here a ternary model was used as opposed to a binary model).

doi:10.1371/journal.pcbi.1003559.g007
Methods

Data set

The data set was obtained from ChEMBL version 14 [31]. For the allosteric set, abstracts and titles of journal articles were searched for keywords (supporting Table S10). For hits both PubMed ID and citation information (primary author, year, journal, volume, and starting page) were kept. From these retrieved records the primary target (based on bioactivity annotation frequency for targets considered in the document) was included along with all compounds annotated on this primary target. As a final step duplicate compounds were removed for each target ID. Herein a distinction was made in the quality of the bioactivity measurement, best measurements (e.g. pKi) were favored over lower quality measurements (e.g. activity comment ‘active’). The background set was retrieved in a similar fashion, but here all document IDs that were not part of the allosteric set were kept. Finally, the balanced non-allosteric set was retrieved from the full non-allosteric set by keeping a random percentage of bioactivities from each L2 target class which was roughly equal in size to the number of bioactivities present in the allosteric set. All data is available on www.gjpvanwesten.nl/allosterism or ftp.ebi.ac.uk/pub/databases/chembl/Allosterism, see supporting Figure S4 for details.

Compound pre-treatment

Compounds were standardized, charged at a pH of 7.4, salts were removed and 2D and 3D coordinates were calculated. All of this was done in Molsoft ICM version 3.7-2d [76].

Compound descriptors

Volume, Polar Surface Area, Molecular weight, and drugLike-ness were calculated in Molsoft ICM, carbon hybridization states were calculated using the Perl molecular toolkit in Pipeline Pilot [76,77]. For partition coefficient (LogP) calculations it has been shown that consensus methods perform well [76], hence the used LogP value was the average of ALOGP calculated in Pipeline Pilot, LogP according to Molsoft ICM and ACD LogP [76,77,79]. Similarly LogD was the average of the pipeline pilot module and ACD LogD, finally solubility was the average of the Pipeline pilot module and logP according to Molsoft ICM, carbon hybridization states were removed and 2D and 3D coordinates were calculated. All of this was done in Molsoft ICM version 3.7-2d [76].

Finally, the rigidity index was an estimation of compound rigidity that was calculated as follows: (Aromatic Bonds fraction)+(1-Rotatable Bonds fraction)+Aliphatic Ring bonds fraction+(1-Single Bonds fraction)+Double Bonds fraction+Triple Bonds fraction+Bridge Bonds fraction)/7;

| Model Predicts A | Model Predicts B |
|------------------|------------------|
| Experiment Measures A | AA | AB | Class A recall (Sensitivity) AA/(AA+AB) |
| Experiment Measures B | BA | BB | Class B recall (Specificity) BB/(BB+BA) |
| Class A precision (PPV) AA/(AA+BA) | Class B precision (NPV) BB/(BB+AB) |

Recall values are calculated over the rows and precision values over the columns. Note that false negatives are missed class A predictions and false positives are missed class B predictions. Hence this can be rewritten as follows:

\[ MCC = \frac{(TP*TN)-(FP*FN)}{\sqrt{(TP+FP)*(TP+FN)*(TN+FP)*(TN+FN)}} \]  

| Numerator: | Denominator: |
|------------|-------------|
| \((AA * BB) \) \( - \) \((BA * AB)\) | \((AA + BA) * (AA + AB) * (BB + BA) * (BB + AB)\) \({1/2}\) |

Target pre-treatment

Target information from ChEMBL (UniProt ID, target classification) was kept as it was defined in ChEMBL. However, when target classification levels were unpopulated the value was replaced with ‘Undefined’.

Machine learning

Models were trained in Pipeline Pilot using the ‘Random Forest’ component. This component uses R-Statistics (version 2.15.0) and the ‘forest’ package [81,82]. For variable importance selection permutation based selection and Gini importance without scaling were used, as recommended by Strobl et al. [83,84]. Important variables were selected based on Pareto optimization of both importance values and class correlation values (e.g. correlation with ‘Allosteric’ class).

Model validation

Validation was performed using 5 different metrics these were: sensitivity (allosteric recall, the fraction of true positives of the total number of allosteric compounds), specificity (non-allosteric recall, the fraction of true negatives of the total number of non-allosteric compounds), positive predictive value (allosteric precision, the fraction of true positives of the total number of compounds predicted to be allosteric modulators), negative predictive value (non-allosteric precision, the fraction of true negatives of the total number of compounds predicted to be non-allosteric modulators), and the Matthews correlation coefficient [85]. Given a confusion matrix were A represents an allosteric modulator classification and B represents non-allosteric modulator classification, Sensitivity is class A recall, and specificity is class B recall, whereas positive predictive value is class A precision and negative predictive value is class B precision (Table 5).

For the MCC equation (1) was used; herein the numerator is the product of the correctly predicted data points minus the product of the incorrectly predicted data points. The denominator is formed by the square root (2-classes) of the total product of all possible sums of correct and incorrectly predicted data points.

\[ MCC = \frac{(TP*TN)-(FP*FN)}{\sqrt{(TP+FP)*(TP+FN)*(TN+FP)*(TN+FN)}} \]  

Note that false negatives are missed class A predictions and false positives are missed class B predictions. Hence this can be rewritten as follows:
The ChEMBL-14 target hierarchy; shown are the first three levels where L0 means the full allosteric versus the full non-allosteric set (protein binding compounds). Descending the hierarchy leads to a finer grained target classification, which eventually culminates in individual proteins (L8). The target distribution overview in the main text is made at target level L2 (red circle).

Substructure frequency analysis

Substructures were obtained using pharmacophore feature class based circular fingerprints (FCFP_6) [63,80]. For all present substructures, substructure frequencies were obtained from the full data set (background frequency), the allosteric set per L2 target (allosteric frequency), and the non-allosteric set per L2 target (non-allosteric frequency). These frequencies were normalized per set (substructure frequency as a fraction of the total substructures per set) to prevent a biased ranking. Subsequently all substructures were ranked based on their normalized frequency.

Enrichment was calculated based on the logarithm of the normalized ranks quotient (between allosteric and background or between non-allosteric and background). These final scores were ranked to obtain the final scored rank.

Supporting Information available

4 supporting figures (Figure S1, S2, S3, S4) and 10 supporting tables (Table S1, S2, S3, S4, S5, S6, S7, S8, S9, S10) that further support the findings are available online. In addition, the datasets, further chemical analyses (per target level), physicochemical property histograms (for L0, L1, and L2), all model training and validation reports, and delimited text files are available online: www.gjpvanwesten.nl/allosterism or ftp.ebi.ac.uk/pub/databases/chembl/Allosterism.

Supporting Information

Figure S1  The ChEMBL-14 target hierarchy; shown are the first three levels where L0 means the full allosteric versus the full non-allosteric set (protein binding compounds). Descending the hierarchy leads to a finer grained target classification, which eventually culminates in individual proteins (L8). The target distribution overview in the main text is made at target level L2 (red circle).

\[
\text{MCC} = \frac{(AA + BB + CC) - (AX + BX + CX)}{\sqrt{(AA + AX) \times (AA + BX) \times (AA + CX) \times (BB + AX) \times (BB + BX) \times (BB + CX) \times (CC + AX) \times (CC + BX) \times (CC + CX)}}
\]
Figure S2  L1 target class distribution of both the allosteric (A) and non-allosteric data (B) sets. Also here the distribution of the target classes differed between the two sets.  

Figure S3  Bar chart of all the mean values for all descriptors in both the allosteric and non-allosteric set of the 7TM2 class (Class B GPCRs). Note that delimited text files are available on www.gjpvanwesten.nl/allosterism or ftp://ftp.ebi.ac.uk/pub/databases/chembl/Allosterrism.  

Figure S4  Layout of the online ftp archive with the extra supporting information.  

Table S1  Physicochemical descriptors used.  

Table S2  Examples of positively enriched allosteric substructures class B GPCR ligands.  

Table S3  Examples of negatively enriched allosteric substructures class B GPCR ligands.  

Table S4  Examples of positively enriched non-allosteric substructures class B GPCR ligands.  

Table S5  Model improvement when including fingerprints in model construction.  

Table S6  Allosteric models for balanced data sets of L0, L1, and L2 groups.  

Table S7  Top 20 property importance for the optimized HIV RT model.  

Table S8  Top 20 property importance for the optimized adenosine model.  

Table S9  Top 17 property importance for the optimized protein kinase B model.  

Table S10  Keywords used to retrieve the allosteric set.  

Acknowledgments  

The authors would like to thank Simone Trubian for the discussions on the work and valuable insights and Francis Atkinson for help in descriptor calculation.  

Author Contributions  

Conceived and designed the experiments: GJPvW AG JPO. Performed the experiments: GJPvW AG JPO. Analyzed the data: GJPvW AG JPO. Contributed reagents/materials/analysis tools: GJPvW. Wrote the paper: GJPvW AG JPO.  

References  

1. Hopkins AL, Groom CR (2002) Opinion: The druggable genome. Nat Rev Drug Discovery 1: 727–730.  
2. Bleicher KH, Bohm HJ, Muller K, Alamine AI (2003) Hit and lead generation: beyond high-throughput screening. Nat Rev Drug Discovery 2: 369–378.  
3. Brown D, Superi-Furga G (2003) Rediscovering the sweet spot in drug discovery. Drug Discov Today 8: 1067–1077.  
4. Overington JP, Ali-Lazikani B, Hopkins AL (2006) How many drug targets are there? Nat Rev Drug Discovery 5: 993–996.  
5. Inming P, Sinning C, Meyer A (2006) Drugs, their targets and the number and number of drug targets. Nat Rev Drug Discovery 5: 821–834.  
6. Cheng AC, Coleman RG, Smyth KT, Cao Q, Soulard P, et al. (2007) Structure-based maximal affinity model predicts small-molecule drugability. Nat Biotech 25: 71–75.  
7. Keseru GM, Makara GM (2009) The influence of lead discovery strategies on the properties of drug candidates. Nat Rev Drug Discovery 8: 203–212.  
8. Lindley JE, Rutter J (2006) Whence cometh the allosterome? Proc Natl Acad Sci U S A 103: 10535–10535.  
9. Lewis JA, Lebois EP, Lindley CW (2008) Allosteric modulation of kinases and GPCRs: design principles and structural diversity. Curr Opin Chem Biol 12: 269–280.  
10. Goody NM, Benkovic SJ (2008) Allosteric regulation and catalysis emerge via a common route. Nat Chem Biol 4: 474–482.  
11. Wang Q, Zheng M, Huang Z, Liu X, Zhou H, et al. (2012) Toward understanding the molecular basis for chemical allosteric modifier design. J Mol Graphics Modell 38: 324–333.  
12. Bacon F (1965) On the nature of allosteric transitions: a plausible model. J Mol Biol 12: 88–118.  
13. Rubin MM, Changeux J-P (1966) On the nature of allosteric transitions: Implications of non-exclusive ligand binding. J Mol Biol 21: 265–274.  
14. Christopoulos A (2002) Allosteric binding sites on cell-surface receptors: novel targets for drug discovery. Nat Rev Drug Discovery 1: 190–210.  
15. Soudijn W, van Wijngaarden I, IJzerman AP (2004) Allosteric modulation of G protein-coupled receptors: perspectives and recent developments. Drug Discov Today 9: 752–760.  
16. Kuzmin T (2007) Collateral efficacy in drug discovery: taking advantage of the good (allosteric) nature of 7TM receptors. Trends Pharmacol Sci 28: 407–415.  
17. Jeffery Conn P, Christopoulos A, Lindley CW (2009) Allosteric modulators of GPCRs: a novel approach for the treatment of CNS disorders. Nat Rev Drug Discov 8: 41–51.  
18. Huang Z, Zhu L, Cao Y, Wu G, Liu X, et al. (2011) ASD: a comprehensive database of allosteric proteins and modulators. Nucleic Acids Res 39: D663–D669.  
19. Fang Z, Gruter C, Rauh D (2012) Strategies for the Selective Regulation of Kinases with Allosteric Modulators: Exploiting Exclusive Structural Features. ACS Chem Biol 8: 58–70.  
20. Russo AA, Jeffrey PD, Patten AK, Massague J, Pavlech NP (1996) Crystal structure of the p27Kip1 cyclin-dependent-kinase inhibitor bound to the cyclin A/Cdk2 complex. Nature 382: 225–231.  
21. Betzi S, Alam R, Martin M, Lubbers DJ, Han H, et al. (2011) Discovery of a Potential Allosteric Ligand Binding Site in CDK2. ACS Chem Biol 6: 492–501.  
22. Pines J (1994) The cell cycle kinases; Semin Cancer Biol. pp. 305–313.  
23. Jeffrey PD, Russo AA, Poyak K, Gibbs E, Harwitz J, et al. (1995) Mechanism of CDK activation revealed by the structure of a cyclin/CDK2 complex. Nature 376: 313–320.  
24. Arkin MK, Wells JA (2004) Small-molecule inhibitors of protein-protein interactions: progressing towards the dream. Nat Rev Drug Discovery 3: 301–317.  
25. Hoare SRJ (2005) Mechanisms of peptide and nonpeptide ligand binding to Class B G protein-coupled receptors. Drug Discov Today 10: 417–427.  
26. Kniazeff J, Przewoz L, Rouard P, Pin J-P, Gouder C (2011) Dimers and beyond: The functional puzzles of class C GPCRs. Pharmacol Ther 130: 9–25.  
27. Gao Z-G, Jacobson KA (2013) Allosteric modulation and functional selectivity of G protein-coupled receptors. Drug Discov Today: Technologies 10: e237–e243.  
28. Kruger FA, Overington JP (2012) Global Analysis of Small Molecule Binding to Related Protein Targets. PLoS Comput Biol 8: e1002333.  
29. De Clercq E (1998) The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection. Antiviral Res 38: 153–179.  
30. Fauvels R, Andries K, Desmyter J, Schols D, Kuikia MJ, et al. (1999) Potent and selective inhibition of HIV-1 replication in vitro by a novel series of TIBO derivatives. Nature 343: 470–474.  
31. Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, et al. (2012) ChEMBL: a large-scale bioactivity database for drug discovery. Nucleic Acids Res 40: D1100–D1107.  
32. Bolton EE, Wang Y, Thiessen PA, Bryant SH (2008) PubChem: integrated platform of small molecules and biological activities. Annu Rep Comput Chem 4: 217–241.  
33. Liu T, Lin Y, Wen X, Jorissen RN, Gilson MK (2007) BindingDB: a web-accessible database of experimentally determined protein–ligand binding affinities. Nucleic Acids Res 35: D198–D201.  
34. Knox C, Law V, Jewison T, Liu P, Ly S, et al. (2011) DrugBank 3.0: a comprehensive resource for ‘Omics’ research on drugs. Nucleic Acids Res 39: D1035–D1041.  
35. Bender A (2010) Databases: Compound bioactivities go public. Nat Chem Biol 6: 309–309.
36. Van Westen GJP, Wegener JK, IJzerman AP, Van Vlijmen HWT, Bender A (2011) Proteochemometric Modeling as a Tool for Designing Selective Compounds and Extrapolating to Novel Targets. Med Chem Commun 2: 16–30.
37. Van Westen GJP, Overington JP (2013) A ligand’s-eye view of protein similarity. Nat Methods 10: 116–117.
38. Bender A, Jenkins JL, Glick M, Deng Z, Nettles JH, et al. (2006) “Bayes affinity fingerprints” improve retrieval rates in virtual screening and define orthogonal pockets. J Chem Inf Model 46: 2445–2456.
39. Keiser MJ, Roth BL, Arumugast E, Emringer PS, Irwin JJ, et al. (2007) Relating protein pharmacology by ligand chemistry. Nat Biotechnol 25: 197–206.
40. Besnard J, Ruda GF, Setola V, Abecasis K, Rodriguiz RM, et al. (2012) Automated design of ligands to polycrylpharmaceutical profiles. Nature 492: 216–220.
41. Kew JNC (2004) Positive and negative allosteric modulation of metabotropic glutamate receptors: emerging therapeutic potential. Pharmacol Ther 104: 233–244.
42. Lane JR, IJzerman AP (2013) Allosteric approaches to GPCR drug discovery. Drug Discov Today: Technologies 10: e219–e223.
43. Li X, Chen Y, Lu S, Huang Z, Liu X, et al. (2013) Towards an understanding of the sequence and structural basis of allosteric proteins. J Mol Graphics Modell 40: 30–39.
44. Pin JP, Knaiezff J, Liu J, Binet V, Goudet C, et al. (2005) Allosteric functioning of dimeric class C G-protein-coupled receptors. FEBS J 272: 2947–2955.
45. Dror RO, Green HF, Valant C, Borhani DW, Valcourt JR, et al. (2013) Structural basis for modulation of a G-protein-coupled receptor by allosteric drugs. Nature 503: 295–299.
46. Harmar A (2001) Family-B G-protein-coupled receptors. Genome Biol 2: 1–10.
47. Murer MO, Kfeld GS (1987) The role of calcium in the development and treatment of osteoporosis. Endocr Rev 8: 377–390.
48. Rubin MR, Bilezikian JP (2003) New anabolic therapies in osteoporosis. Endocrinol Metab Clin 32: 285.
49. Holz GG, Chepurny OG (2003) Glutacoin-like peptide-1 synthetic analogs: new therapeutic agents for use in the treatment of diabetes mellitus. Curr Med Chem 10: 2471.
50. Hopkins AL, Groom CR, Alex A (2004) Ligand efficiency: a useful metric for lead selection. Drug Discov Today 9: 845–851.
51. Abad-Zapatero C (2007) Ligand efficiency indices for effective drug discovery. Expert Opin Drug Discov 2: 469–488.
52. Abad-Zapatero C, Petrus O, Wass J, Bento AP, Overington J, et al. (2010) Ligand efficiency indices for an effective mapping of chemico-biological space: the concept of an atlas-like representation. Drug Discov Today 15: 804–811.
53. Jensen AA, Spalding TA (2004) Allosteric modulation of G-protein-coupled receptors. Eur J Pharm Sci 21: 407–420.
54. May LT, Leach K, Sexton PM, Christopoulos A (2007) Allosteric Modulation of G Protein-Coupled Receptors. Annu Rev Pharmacol Toxicol 47: 1–31.
55. Heinemann M, Sauer U (2010) Systems biology of microbial metabolism. Curr Opin Microbiol 13: 337–343.
56. Link H, Koschanowski K, Sauer U (2013) Systematic identification of allosteric protein–metabolite interactions that control enzyme activity in vivo. Nat Biotechnol 31: 357–361.
57. Sykes DA, Dowling MR, Charlton SJ (2009) Exploring the Mechanism of Agonist Efficacy: A Relationship between Efficacy and Agonist Dissociation Rate at the Muscarinic M3 Receptor. Mol Pharmacol 76: 543–551.
58. Guo D, Mulder-Krieger T, IJzerman AP, Heiteman LH (2012) Functional efficacy of adenosine A2A receptor agonists is positively correlated to their receptor residence time. Br J Pharmacol 166: 1046–1059.
59. Vilains M, Zweemer AJM, Yu Z, de Vries H, Hilger JM, et al. (2013) Structure-Kinetic Relationships—An Overlooked Parameter in Hit-to-Lead Optimization: A Case of Cyclopentylaminides as Chemokine Receptor 2 Antagonists. J Med Chem 56: 7706–7714.
60. Stockton JM, Birdsell NJ, Burgen AS, Hulme EC (1983) Modification of the binding properties of muscarinic receptors by gallamine. Mol Pharmacol 23: 551–557.
61. Hu Y, Bajajrat J (2013) What is the Likelihood of an Active Compound to Be Promiscuous? Systematic Assessment of Compound Promiscuity on the Basis of PubChem Confirmatory Bioassay Data. AAPS J: 1–8.
62. Strohl C, Boulester A-L, Zeileis A, Hothorn T (2007) Bias in random forest variable importance measures. In: Statistical Learning and Computational Statistics. Springer, Berlin.
63. Matthews BW (1975) Comparison of the Predicted and Observed Secondary Structure of t4 Phage Lysozyme. Biochim Biophys Acta 405: 442–451.