

Scaffold-Based Analytics: Enabling Hit-to-Lead Decisions by Visualizing Chemical Series Linked Across Large Datasets

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Abstract

We present a method for visualizing and navigating large and diverse chemical spaces, such as screening datasets, along with their activities and properties. Our approach is to annotate the data with all possible scaffolds contained within each molecule using an exhaustive algorithm developed at NCATS. We have developed a Spotfire visualization, coupled to a fuzzy clustering approach, that is used to drive the hit triage process. Progression decisions can be made using aggregate scaffold parameters and data from multiple datasets merged at the scaffold level. This visualization easily reveals overlaps that help prioritize hits, highlight tractable series and posit ways to

combine aspects of multiple hits. The SAR of a large and complex hit is automatically mapped into all constituent scaffolds making it possible to navigate, via any shared scaffold, to all related hits. This scaffold “walking” helps address bias toward a handful of potent and ligand-efficient molecules at the expense of coverage of chemical space. We compare the NCATS scaffold generation method with published screening triage methods such as nearest-neighbor clustering, data-driven clustering and scaffold networks. Similarities and differences in the methods are evaluated qualitatively and statistically. The workflow of a Spotfire visualization used in combination with fuzzy clustering and structure annotation provides a novel view of large and diverse datasets. This allows teams to effortlessly navigate between structurally related molecules and enriches the population of leads considered and progressed in a manner complementary to established approaches.

Introduction

The advantage and disadvantage of high throughput screening (HTS) campaigns is the large amount of data that is generated. While the value of large scale HTS has been debated,¹ the massive structure-activity datasets generated create a challenge in identifying truly active compounds and their analogs and weeding out false positives. The process of reducing HTS datasets from hundreds of thousands of compounds to a few thousand or hundred active series is termed triaging. Over the last twenty years many approaches to HTS triaging have been described which include activity based thresholds,² similarity to known actives,³ enrichment based approaches,^{4,5} ranges of physicochemical properties,⁶ crowdsourcing⁷ and removal of promiscuous or otherwise undesirable chemotypes.⁸ See Shun et al. and Langer et al. for a review of HTS triage approaches.

A key challenge in the triage step is to identify structure-activity series - sets of compounds with similar or analogous structures that exhibit a spectrum of activity, including lack of activity. Identifying such subsets allows one to have some confidence in the presence of a

structure-activity relationship amongst the active compounds which enables a more efficient exploration of the chemical space around the selected hits. A good review of computational methods to extract SAR from screening datasets can be found in Wawer et al..

Given that a SAR series is, ideally, defined in terms of a core structure along with various decorations, the first step in the triage process is to identify these core structures, termed scaffolds. Usually this starts by decomposing the structures in the screening collection, either exhaustively or else using one of the many methods to decompose structures into fragments such as the Bemis-Murcko^{12,13} or RECAP methods.¹⁴ These methods lead to a large number of fragments ranging from trivial ones such as a benzene ring to very complex multiring structures. Thus a key step involves identifying the relevant set of scaffolds and their associated compounds. This can be challenging since a given compound can contain multiple scaffolds and which scaffolds are deemed relevant can be a subjective decision.¹⁵

In this work we present a HTS triage workflow based on navigating the scaffold-activity landscape of a screening collection using a fuzzy clustering method to group compounds based on scaffold membership. The workflow includes methods to visualize the activity landscape as well as methods to explore different regions of chemical space via shared scaffolds. We also highlight the efficiency gains obtained by using pre-computed scaffold associations rather than performing substructure searches on the fly.

Related Work

While there are many ways to generate a set of scaffolds from a compound collection, a key step is to identify a relevant subset of them or else aggregate them in a way that leads to a useful clustering of active and inactive compounds. While the term “useful” is rather subjective, it is easy to identify cases that are not useful. Thus, 5- or 6-member undecorated rings are likely not useful since they will occur in the majority of compounds in a screening collection. At the other extreme, large, extended scaffolds that are associated with very few compounds are also likely not useful.

As a result, many approaches to scaffold aggregation have been described. A natural approach is to consider a hierarchical aggregation. The Scaffold Tree¹⁶ and Scaffold Network¹⁷ define a hierarchical decomposition from more specialized larger scaffolds to more inclusive smaller scaffolds. While the Scaffold Tree splits each larger scaffold in exactly one way into two scaffolds with fewer rings, the Scaffold Network performs an exhaustive decomposition into all possible smaller scaffolds with fewer rings. Since some subscaffolds are shared with neighboring scaffolds, this produces a network or graph rather than a tree. The Scaffold Network Generator program¹⁸ is a fast, easy-to-use public command line implementation of Scaffold Networks that can scale to millions of compounds via aggregation of scaffolds from split datasets and parallel execution. Harper et al. use exhaustive enumeration to find all Bemis-Murcko like frameworks in each molecule, and then recursively retain those frameworks with highest aggregate activity and remove molecules that contain them until a threshold is met, yielding a set of disjoint frameworks. Other methods have used multiple common substructure (MCS), first proposed for finding protein structural similarity,²⁰ for example Quintus et al. and the ChemAxon product LibraryMCS.

To overcome the effect of small variations in heteroatoms (eg. O to S) mapping otherwise similar molecules to different scaffolds, generalized or consolidated scaffold representations have been proposed, such as the Reduced Graph,²² the Bemis-Murcko scaffold¹³ and the topological or 2D pharmacophore.²³ Bemis-Murcko like frameworks¹⁹ are generalized Bemis-Murcko scaffolds where atom types and/or bond orders may be retained.

Multiple scaffolds if present in a dataset can be inferred from the scaffold tree decomposition.²⁴ However in practice, the thresholds used by Clark and Labute miss common scaffolds in HTS-like diverse chemical compound sets. Bandyopadhyay used a common fragment decomposition plug-in for SAREport in order to find and export scaffolds for diverse datasets, such as the GSK Natural Products Set.²⁶ This approach assigns at most one scaffold for each molecule, based on the order they are selected by the user from a prioritized list.

Assigning each molecule to a single group partitions the dataset into disparate, non-

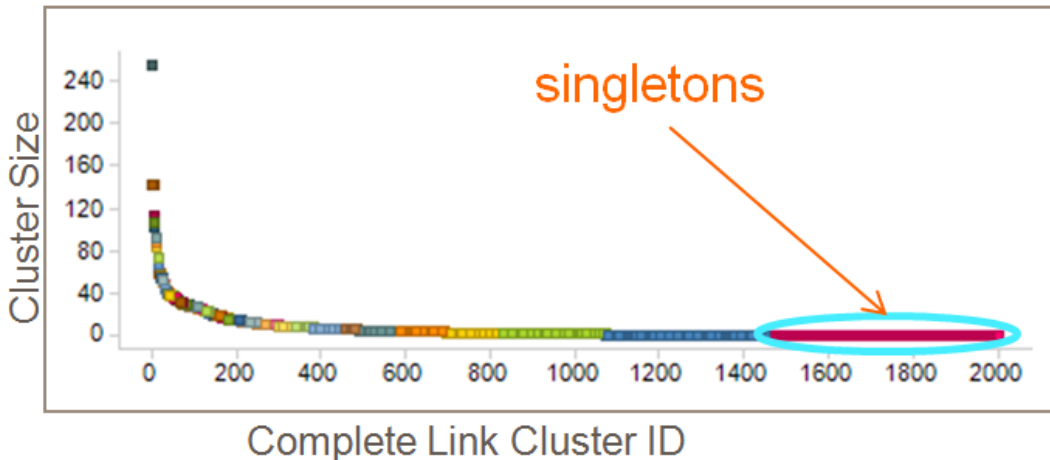


Figure 1: Singletons in a complete-linkage clustering of the TCAMS dataset.

overlapping groups (also termed a hard clustering). This is also the way most fingerprint-based agglomerative clustering methods such as sphere exclusion, single and complete linkage clustering work.²⁷ On the other hand, it may be the case that a molecule could well be assigned simultaneously to multiple clusters, depending on the structural features present. Traditional hard clustering methods will not allow this and as a result will tend to assign such molecules to their own cluster, thus identifying them as singletons. Such a result can be observed in real chemical datasets as well – for example, the complete-linkage clustering of the TCAMS dataset^{28,29} has nearly 25% of the 2000 clusters containing just one compound, as shown in Figure 1.

In contrast to these partitioning clusters, the methods described here rely on fuzzy clustering, where a molecule may be assigned to multiple clusters. Fuzzy clusters have been rarely used in cheminformatics (for example^{30,31}), perhaps because they are hard to visualize and navigate. In this work we provide an intuitive visual framework that we believe can help end users easily navigate a diverse chemical dataset described by a fuzzy clustering of multiple overlapping scaffolds per molecule.

Datasets

The datasets used to illustrate and visualize our methods were picked to represent the kinds of screening datasets we expect the method to be used on in practice. For example, the TCAMS dataset²⁸ consists of 13.5k diverse hits from an antimalarial screen at GSK, along with pIC50 against a susceptible strain of the malarial parasite (3D7), percentage inhibition against a resistant strain (DD2), Hep G2 hepatotoxicity, a few physical chemical properties (e.g., molecular weight, aromatic ring count, cLogP), and Inhibition Frequency Index (IFI, a measure of promiscuity defined as the percentage of screens in which a molecule inhibits over 50%,³²).

The in-house GSK kinase dataset shown (“Kinase X”), by contrast, is perhaps less diverse but chosen to illustrate the power of this approach in joining and merging datasets from multiple screens, combining their SAR to design hybrid molecules, and making inferences about unknown activity in one screen based on known activity in another screen.

Next, we describe several methods we have assembled as part of our workflow that enable multiple scaffolds to be assigned to each molecule and easy navigation between molecules related by these scaffolds.

Dataset Preprocessing

The typical dataset under consideration is available as a comma-separated text file (CSV), whereas most of the scaffold decomposition methods described expect MDL SD-files. To convert CSV to SDF while preserving non-molecule fields as SDF properties, we have created a simple Pipeline Pilot workflow, but this can also be done via standard cheminformatics toolkits such as JChem or RDKit. Prior to SDF conversion, activity or property columns that are not to be aggregated at the scaffold level should be deleted from the CSV file, in order to speed up the analysis and aggregation. There are some further quirks to pre-processing datasets for the NCATS R-group tool that will be described in the Supplementary Material in section S3.

Partitioning Method: Complete Linkage Clustering

We retain the default method used at GSK to visualize groups of molecules in our visualizations just for comparison purposes. This method produces an output file in which the unique cluster ID (CLink) and number of other molecules in the same cluster (N_Clink) are added as additional fields to the original dataset. Complete linkage chemical clustering is described further in several references such as Downs and Barnard, Jain.

Fragmentation Method: NCATS R-Group Tool

The NCATS R-group analysis tool (<https://tripod.nih.gov/?p=46>) was developed to automatically and exhaustively generate R-group tables from a dataset using all scaffolds, defined as chemical substructures shared by two or more molecules. The scaffolds are defined as molecular fragments generated by exhaustive enumeration of all possible combinations of the Smallest Set of Smallest Rings (SSSR), as described at <https://tripod.nih.gov/?p=160>. For a molecule with k SSSR, the maximum possible number of such scaffolds is $2^k - 1$; however the actual number is usually much lower due to symmetry and additional constraints (e.g., reactivity, synthetic accessibility). Briefly, the fragments are generated based on the following rules:

1. Generate Bemis-Murcko framework by iteratively pruning all foliage except for carbonyl (or any terminal double bond).
2. Exhaustively enumerate all possible combinations of ring system. This is achieved by iteratively breaking non-aromatic bonds and keeping unique set of ring systems.
3. Each fragment generated should be a valid substructure of the parent molecule. This constraint is approximated by ensuring the fingerprint for the fragment is a subset of the parent molecule.

As an example, in Figure 2(b) we see the five scaffolds that were generated from the molecule shown in Figure 2(a) from the TCAMS dataset.

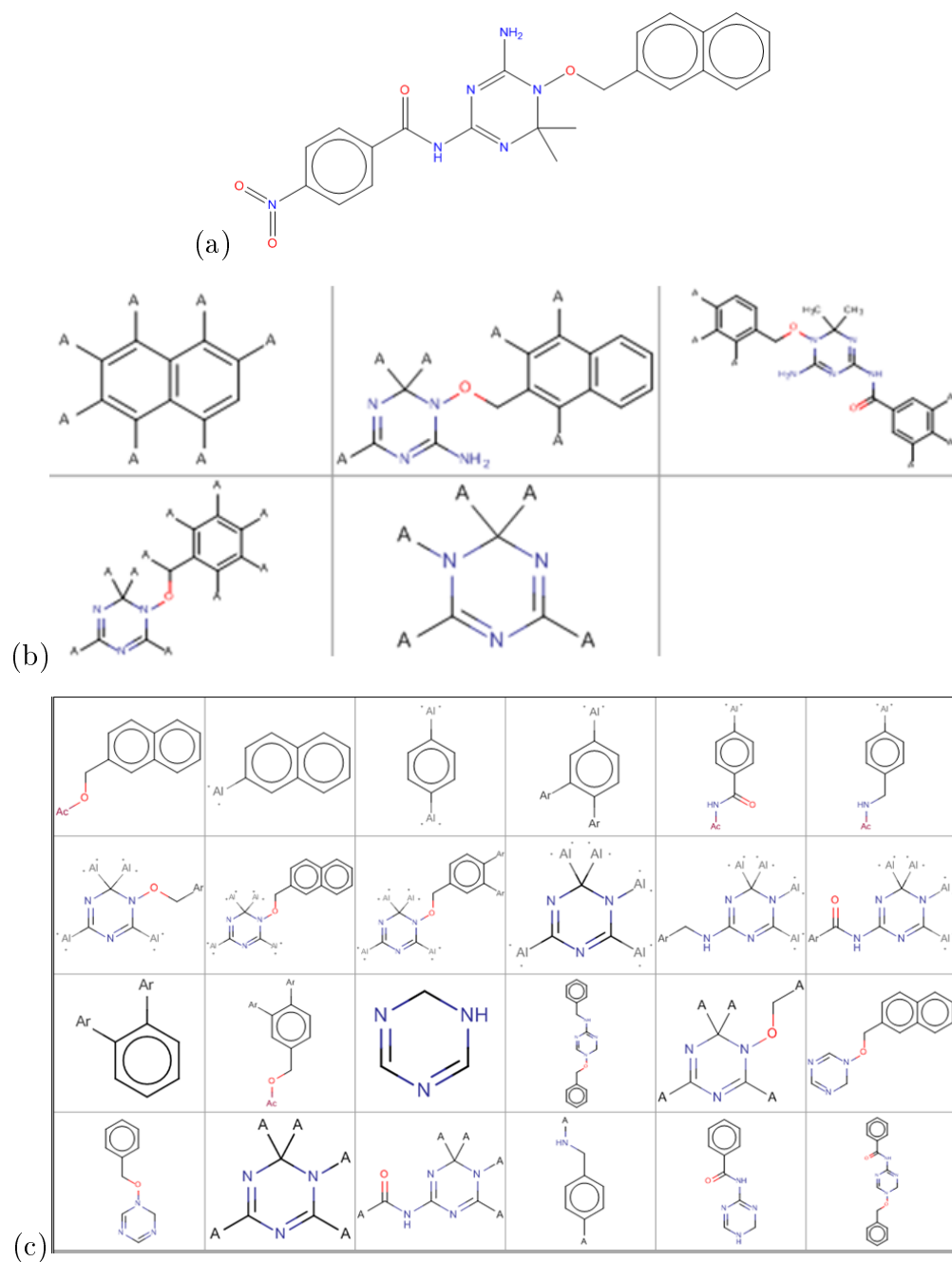


Figure 2: Scaffold Decompositions for a molecule (a) from the TCAMS dataset with PubChem Compound ID 536182. (b) 5 scaffolds from NCATS R-Group Tool; (c) 24 GSK frameworks – first 21 Bemis-Murcko Like and last 3 in the bottom row RECAP.

The original NCATS R-group tool focused on scaffold decomposition and the subsequent generation of R-group tables for each scaffold. Since then it has been extended to support scaffold hopping as well as providing contextual data (e.g., literature references, activity summaries) and network visualizations of scaffold relationships. All results from this study were generated in version 8 of the R-group tool, (<http://tripod.nih.gov/ws/rgrouptool8.jar>). The latest version available at time of writing is (<http://tripod.nih.gov/ws/rgrouptool11.jar>), which has been tested and produces substantially similar output.

When running from the command-line, ensuring 16G of memory (via `-Xmx16G`) enables datasets of upto 40k compounds with a handful of numeric activity columns to be analyzed without running out of memory. The scaffolds along with R-group tables for each scaffold can be exported in a set of TSV files or a single JSON file. In the current work we employed the TSV format which is described in detail in Supplementary Tables S1 & S2.

Fragmentation Method: GSK Frameworks

The R-group Tool described above uses the NCATS implementation of Molecular Frameworks to generate scaffolds. GSK’s implementation of Molecular Frameworks is subtly different, and its use has been described in Harper et al.. The two types of framework we retain for this study are Bemis-Murcko-like¹³ (with atom and bond orders retained) and RECAP.¹⁴ Other fragmentation methods described by Harper et al. such as reduced graphs and classic Bemis-Murcko scaffolds (without atom types and bond orders) were skipped for the purposes of comparison; however there is no reason these could not be included in the technique we are proposing.

The input for the GSK frameworks code is a comma separated text file with molecules encoded in a SMILES field. The code was modified by adding scripts to export the fuzzy clusters in a tabular format rather than prioritize them into mutually exclusive scaffolds as in Harper et al.. This step produces a file similar to the R-group decomposition format

described for the NCATS R-group tool in Section , including the following key columns: framework ID, framework SMILES, molecule ID, SMILES and properties/activities. There are no R-group columns simply because this is not a default computation in the GSK frameworks code.

The GSK frameworks found within the same molecule from TCAMS are shown in Figure 2(c). The reader will observe several differences from the R-group tool: there are more scaffolds found, some clipped in the middle of a linker rather than at a ring, and some redundancy between multiple scaffolds. Also the current implementation does not convert or unify tautomers among scaffolds, again leading to larger numbers of scaffolds.

Further details on how to set up and run the GSK frameworks code are provided in the Supplementary Material Section S4.

Methods: Data Integration and Visualization in Spotfire

Next, we describe how tabular scaffold output generated using the NCATS R-group tool and other comparable methods is integrated into Spotfire, our visualization tool of choice at GSK.

Data Table Generation and Linking

Figure 3 shows how the data tables output by the scaffold generation methods considered here are layered onto the primary data table in Spotfire. This primary data table is usually a direct import of tabular molecule and activity data generated at GSK or available from public datasets. What gets added is by and large similar, following the schema “Molecule \rightarrow Scaffold (including Annotation) \rightarrow Related Molecules” shown in the figure. For each Molecule in the dataset, we connect it to every Scaffold/Framework/Cluster it contains, and then to every other molecule containing any of these Scaffolds/Frameworks/Clusters. Slight differences for each individual method are detailed in the Supplementary Material, Section



Figure 3: Detailed schematic on how the output from clustering and fragmentation methods are set up as data tables and linked together with the main dataset in Spofire. Right inset: schematic color-coded view of the scaffold-walking navigation that is loosely followed in this diagram.

Visualization of Molecules, Scaffolds and Related Molecules

We next describe a minimalistic user interface for exploring the network of molecules, the scaffolds they contain and related molecules that contain the same scaffolds. This interface consists of the following elements:

- **Main Window:** Views defined that allow one to explore the Primary Data Table (with no scaffold information) in the most useful way for each dataset. In the canonical example, key activity, selectivity, ligand efficiency and molecular properties may be highlighted on the X, Y, shape, size and color axes on a scatter plot. This window is not illustrated in the interests of space, and since the reader familiar with screening datasets may already have seen a similar view.
- **Related Molecules Tab:** The purpose of this tab is to implement the Scaffold Walking navigation described briefly earlier. The setup is described for the NCATS R-group Tool decomposition, though this tab applies to and can be set up analogously for any other decomposition. The tab consists of two visualizations, illustrated for the TCAMS dataset in Figure 4(a):

The first one is a miniature version of the Main window, allowing the user to select (in Spotfire, mark) molecules of interest without flipping over to the Main tab. Doing so drives one of the following two Details Visualizations on the RGdecomp table, showing only molecules from the scaffolds contained in the marked molecule, i.e., Related Molecules:

Scaffold Trellis: This scatter plot is trellised by Scaffold ID and ideally displays the same properties on the axes as the Main visualization above it. An example is shown in Figure 7. The trellis allows us to break up the SAR for each constituent scaffold individually, identifying promising scaffolds and substituting unproductive ones as we

will describe in the Discussion. The trellis visualization suffers from redundancy: the same molecule occurs in multiple trellis panels and the only way to link them is by X and Y coordinates, by observing groups of points that are laid out similarly across multiple trellis panels. Though some of our users still prefer this approach, we now describe a newer solution that better leverages Spotfire’s capabilities.

Scaffold Pies: Instead of using a trellis, the Marker shape is changed to Pies, with Colors (which map to pie sectors) by Scaffold ID, and sectors sized by the Count of molecules in each scaffold. The result is illustrated in Figure 4(a). This plot shows only one point per related molecule but one sector for each scaffold it shares with the parent molecule. As described in Section , this lets the user quickly and visually home in on key substructures that are important or unimportant for activity.

- **Scaffolds and R-groups Tab:** This tab, currently specific to the NCATS R-group tool method for generating scaffolds, contains two visualizations, as illustrated for the TCAMS dataset in Figure 4(b)–(c):

The first is a scatter plot display of the Scaffolds table, displaying scaffolds Complexity and Count and aggregate activity of each scaffold either on the axes or using the Size and Shape dimensions. Here scaffolds of lesser interest (for example with low complexity or count) can be identified and tagged to remove them from the analysis. Conversely, scaffolds of high interest, for example with many active members or high aggregate ligand efficiency, may be tagged into separate categories.

The second plot is an R-group table, i.e., a Table view of the RGdecomp table limited to data records that have been marked, i.e., molecules that lie in scaffolds currently marked. The table is sorted first by scaffold and then by primary activity, and molecular fields such as Scaffold SMILES, Molecule SMILES and R-groups $R_1..R_n$ are rendered using an appropriate depiction package - at GSK this is JChem. This table may be exported to Excel as an on-the-fly R-group table of the scaffolds of interest.

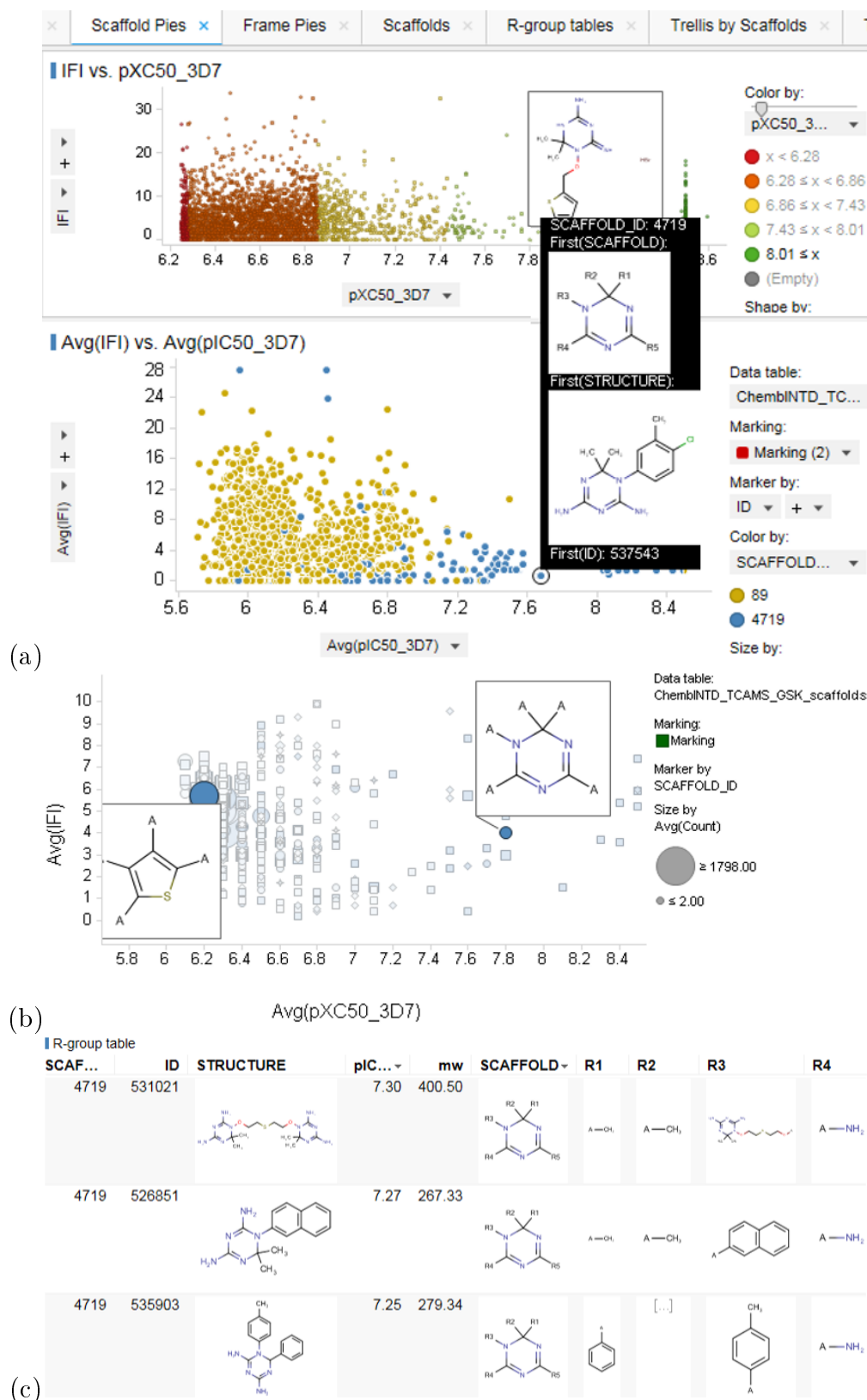


Figure 4: Elements of the minimal Spotfire interface we developed for Scaffold-Based Analytics: (a) Related Molecules page, with molecule selection in the top panel and related molecules viewed as one pie sector per scaffold shared; (b) Aggregate plot of scaffold statistics (with often a detailed drill-down into the selected scaffold); (c) R-group table on selected scaffolds, sorted by scaffold ID and decreasing pXC50 or other desired properties.

Alternative visualizations are possible and we describe some of them in the Supplementary Material, Section S7. In addition, we discuss several Spotfire tricks that are instrumental in making our visualization useful to the chemist or biologist user.

Statistical Comparison of Scaffold Generation Methods

In this section we define the statistical methods used to compare the scaffold generation methods in the context of annotating molecules in a screening dataset with multiple, possibly overlapping scaffolds (as opposed to partitioning the set of molecules into non-overlapping categories, a.k.a. clustering). The code to implement these methods is released as part of the Supplementary Material in Section S8.

The similarities and differences between non-overlapping clusterings have been analyzed in the literature, and one example is the method of Torres et al., who provide a similarity measure between outputs of two partitioning clustering methods. They use Jaccard’s similarity coefficient S_{ij} , defined as the ratio of intersection size to union size of two groups i and j in the clustering. This coefficient, computed pairwise and summed, yields a simple similarity measure between two clusterings X and Y with m and n clusters, respectively:

$$Sim(X, Y) = \sum_{i \leq m, j \leq n} S_{ij} / \max(m, n) \quad (1)$$

We adapt this method for SAR analysis using overlapping scaffolds, where the question on the minds of chemists is twofold: what are the fragments in active/desirable molecules, and which other molecules share these fragments? Given two fragmentation schemes, A and B , we can evaluate for any molecule how many other molecules share fragments using A or B , and thus can be found using fragmentations A alone, B alone, A and B , or A excluding B . Using these methods we can evaluate the overall similarity of the two fragmentation schemes, and also independently score the usefulness of A and B to connect compound(s) of interest to related molecules.

For any compound C and ontology A , define the structure group of C under A , C_A as the set of compounds that share fragments from A with compound C . Similarly define the structure group of C under B , C_B as the set of compounds that share fragments from B . The Common Proportion (CP) for compound C is then defined as the ratio of the number of compounds common to C_A and C_B to the total number of unique compounds contained in C_A and C_B :

$$CP_{A,B}(C) = \|C_A \cap C_B\| / \|C_A \cup C_B\| \quad (2)$$

Similar statistics can be defined to rank the usefulness of an individual fragmentation given others. The Proportion of Information PI_A calculates the proportion of compounds reachable from A and B that would be reachable from A alone:

$$PI_A(C) = \|C_A\| / \|C_A \cup C_B\| \quad (3)$$

In contrast, the Proportion of Information Unique to A , PIU_A uses the set difference between C_A and C_B to get at the question: if I have B , do I still need A ?

$$PIU_A(C) = \|C_A \setminus C_B\| / \|C_A \cup C_B\| = 1 - PI_c(B) \quad (4)$$

When comparing one fragmentation method against another, we often see that one method utilizes a vastly greater number of shared fragments than the other in order to connect compound C to a very similarly sized structure group. To capture this tendency and reward methods that connect molecules to related ones efficiently rather than exhaustively, we define a Fragment Efficiency measure as follows. Let $frag_A(C)$ be the set of fragments of compound C in ontology A that connects C to its structure group C_A . Similarly define $frag_B(C)$ for ontology B . Then:

$$FragEff_A(C) = \|C_A\| / \|frag_A(C)\| \quad (5)$$

These statistics can be averaged over all compounds in a dataset to yield the Average Common Proportion (ACP), Average Proportion of Information (API), Average Proportion of Information Unique to A (APIU) or Average Fragment Efficiency (AFE). Other statistics can also be applied to the distribution of *CP*, *PI* or *PIU* to characterize the dataset and overlapping scaffolds used to characterize it.

Our methods extend the similarity score of Torres et al. to overlapping clusters by using a set of clusters derived from them, defined as follows:

- Replace the original clusters by new clusters, one for each item in any cluster (in this case, any compound in the dataset).
- The cluster for Compound *C* contains all compounds that appear with Compound *C* in any cluster, i.e., C_A .

Results

Here we illustrate some of our key findings and use cases on a few datasets.

Use Case: Scaffold Progression and Prioritization

Aggregate statistics such as maximum, minimum, mean and standard deviation, computed at a per scaffold level, may be useful in prioritizing scaffolds. For example, Figure 5 shows the six scaffolds contained within a tricyclic molecule from TCAMS (Molecule 2, CID: 533945) ranked by average activity and IFI. This ordering may be used to determine which substructures are most important for the molecule’s activity, and use this information to design or test further compounds.

For the in-house kinase dataset, the program team were looking for hit series with activity in a range of orthogonal on-target assays, selectivity against a mutant counter-screen, and good properties to predict developability. We aggregated the activity in each of the primary

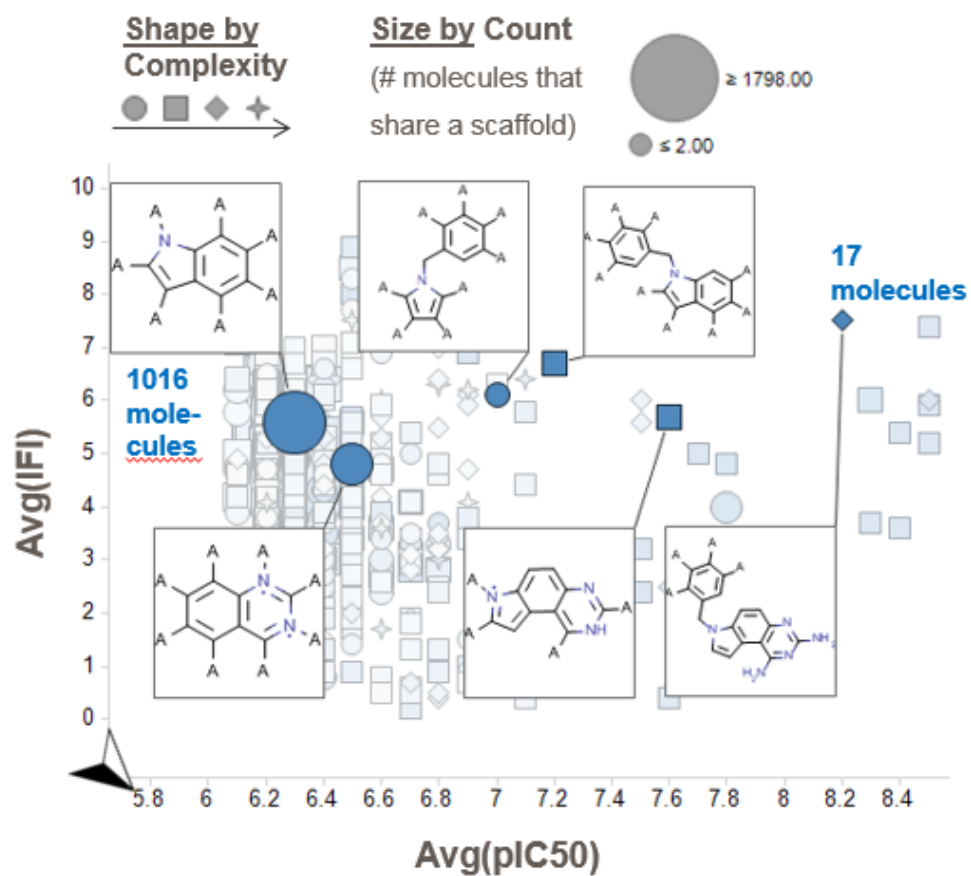


Figure 5: All 5000 scaffolds from the TCAMS dataset ranked by average pIC₅₀ in the *P. falciparum* 3D7 strain and Inhibition Frequency Index. The six scaffolds contained in Molecule 2 (CID: 533945) are shown in increasing order of average

assays, selectivity, and Property Forecast Index (PFI³⁵), using the mean values as filters to identify promising scaffolds. A plot of aggregate selectivity vs. PFI is shown in Figure 6(a).

Once a promising scaffold was identified, one could drill down with a single click into detailed activities and properties of the molecules containing the scaffold, to decide whether to pursue the series further. For example, the scaffold with ID 2988, marked in Figure 6(a) has on the aggregate good selectivity (1.4 log units) and also low PFI (6.7). The activity profiles for 8 molecules sharing this scaffold are shown in Figure 6(b). Clear differentiation is observed between an orthogonal suite of native protein assays and the mutant assay, marking the scaffold as a good one to explore making additional molecules.

To conclude, we have observed how scaffold-based analytics allows us to make decisions based on the aggregate (average, SD, median, min, max, ...) properties of molecules in scaffolds. An entire scaffold may be rejected or prioritized at a time instead of keeping track of individual molecules. It is important to note that rejecting a scaffold does not reject all molecules containing that scaffold - otherwise rejecting the benzene ring would remove a large percentage of valid leads. Also at any time we are able to drill down into the data for individual compounds in interesting scaffolds, whether in an R-group table or a custom plot, and incorporate that into our prioritization.

Use Case: Dataset Fusion and Hybridization

In the previous section and Figure 6, we noted that the 8 molecules sharing scaffold 2988 came from two datasets, viz. HTS hits from 2012 and 2014. This is significant since the 5 molecules found during the 2012 campaign were not prioritized since they did not jump to the top using visual inspection, clustering or other methods used to triage the hits – this Scaffold-Based Analytics method was not available. However, when the hits from both screens were combined and analyzed with this method in 2014, the pattern of activity, selectivity and properties that made these desirable hits to pursue was automatically found and readily flagged.

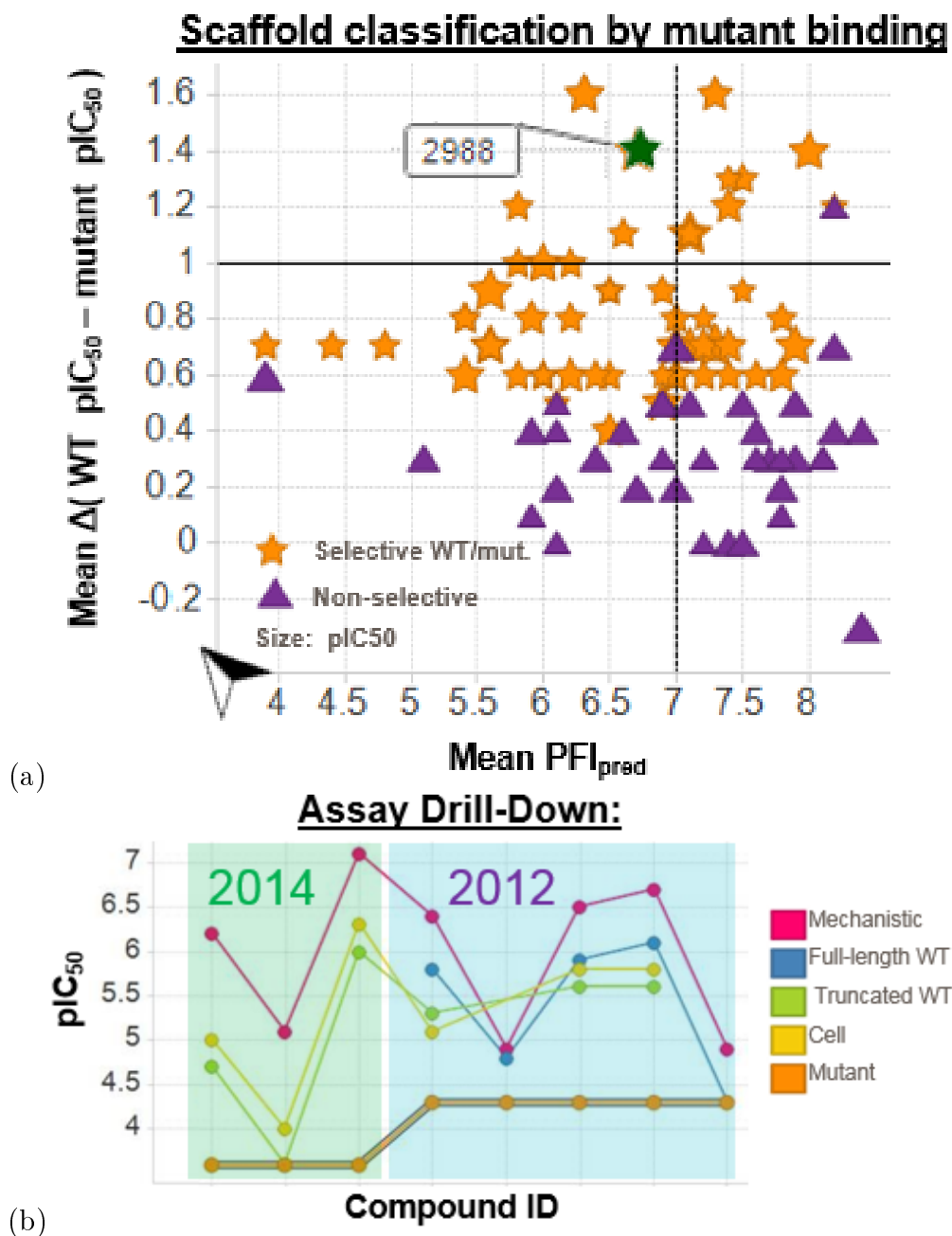


Figure 6: (a) Aggregate scaffold statistics for molecules from the Kinase X dataset, with average selectivity against a mutant assay in log units on the Y-axis and average PFI (Property Forecast Index) on the X-axis. Scaffolds are tagged as selective or non-selective after exploring their detailed properties. (b) Detailed properties of 8 molecules that share scaffold 2988 (structure not shown), split over two HTS datasets from 2014 and 2012. Lines are color-coded by assay type, and the mutant assay is shown in orange across the bottom of the graph.

In this way, Scaffold-Based analytics may be used to combine multiple datasets using the scaffold as the common unit of comparison and merging, with dataset labels identifying molecules in the resulting merged dataset. If the datasets have comparable activities, as the Kinase X HTS datasets did, aggregate statistics are easy to compute. If they do not, e.g., if one dataset has pIC_{50} and others primary screening activity or a different measure of desirability, normalized activities may be computed to enable aggregation at the scaffold level.

Sometimes one of the datasets does not contain activities at all, e.g., in the case of virtual molecules from an Encoded Library Technology screen, or for molecules from a vendor catalog that are yet to be ordered and assayed. In Figure 7 we show an example where two computed properties, Molecular Weight and PFI were used to identify promising virtual molecules from the ELT dataset to make, and untested molecules from the FBDD (Fragment-Based Drug Design) dataset to test.

Use Case: Scaffold Walking Navigation

Scaffold Walking is our term for navigating from molecule(s) through scaffolds (implicitly) to Related Molecules. This contrasts with Scaffold Hopping, which is usually defined as a complete replacement of a scaffold by another 2D-dissimilar but 3D-similar or bioisosteric scaffold. Scaffold Walking is meant to be a gradual change to the molecule, at each step retaining at least one element of its maximal Murcko scaffold (i.e., at least one among the multiple scaffolds it shares with other molecules in the dataset). In the process the SAR gets deconvoluted in terms of these scaffolds, allowing us to determine visually both the most essential scaffolds in a molecule and the best Related Molecules containing them.

As an example, consider Molecule 1 (PubChem CID: 536182) in Figure 8, as a hit that we want to explore SAR of and optimize. This molecule contains 5 scaffolds as determined by the NCATS R-group tool. Using the Scaffold Pie visualization, we observe that the naphthyl scaffold (#978, cyan) is by itself only moderately active. The dihydrotriazine (#4719, blue)

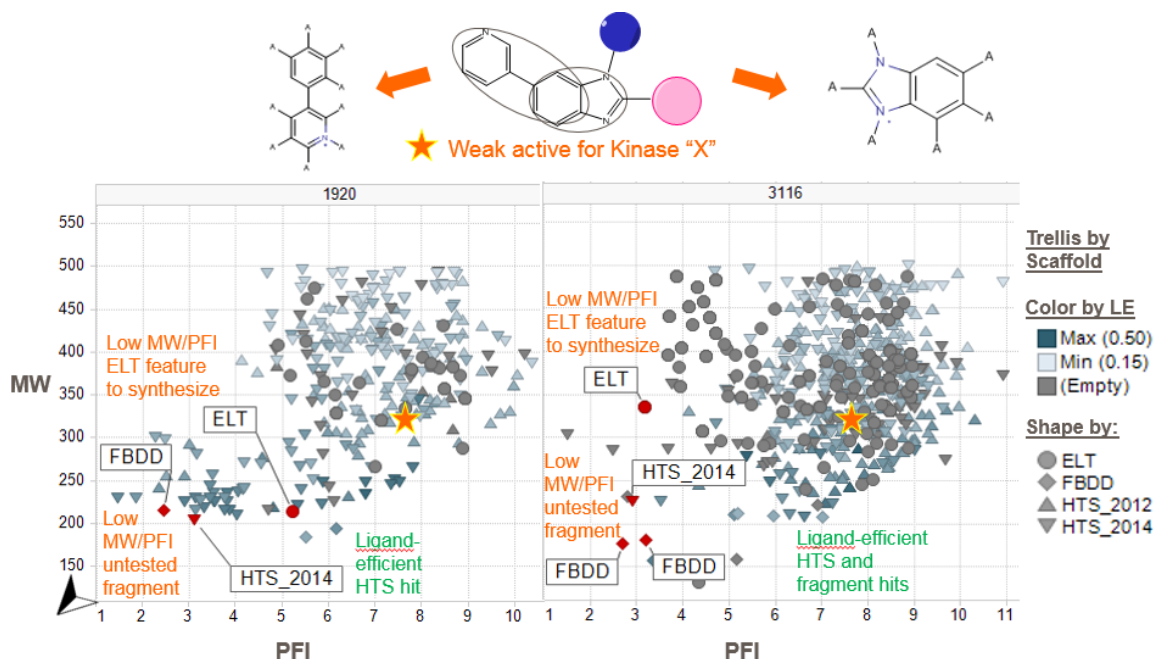


Figure 7: Two scaffolds with ID 1920 (pyridophenyl) and 3116 (benzimidazole) shown in a trellised plot of molecules related to a weak active in the Kinase X 2014 HTS screen. The axes of the plots are computed properties, MW and PFI, to allow molecules without measured activity or virtual molecules not yet synthesized to be included in the plot. The compass arrow device points in the direction of improved properties – lower MW and PFI. Interesting related molecules are labeled on the plot, including untested fragment-like molecules from the FBDD dataset, virtual molecules from the ELT dataset, and another ligand-efficient hit from the HTS2014 dataset, all with low MW/PFI. The unmeasured and unmade molecules are good candidates for future synthesis and testing.

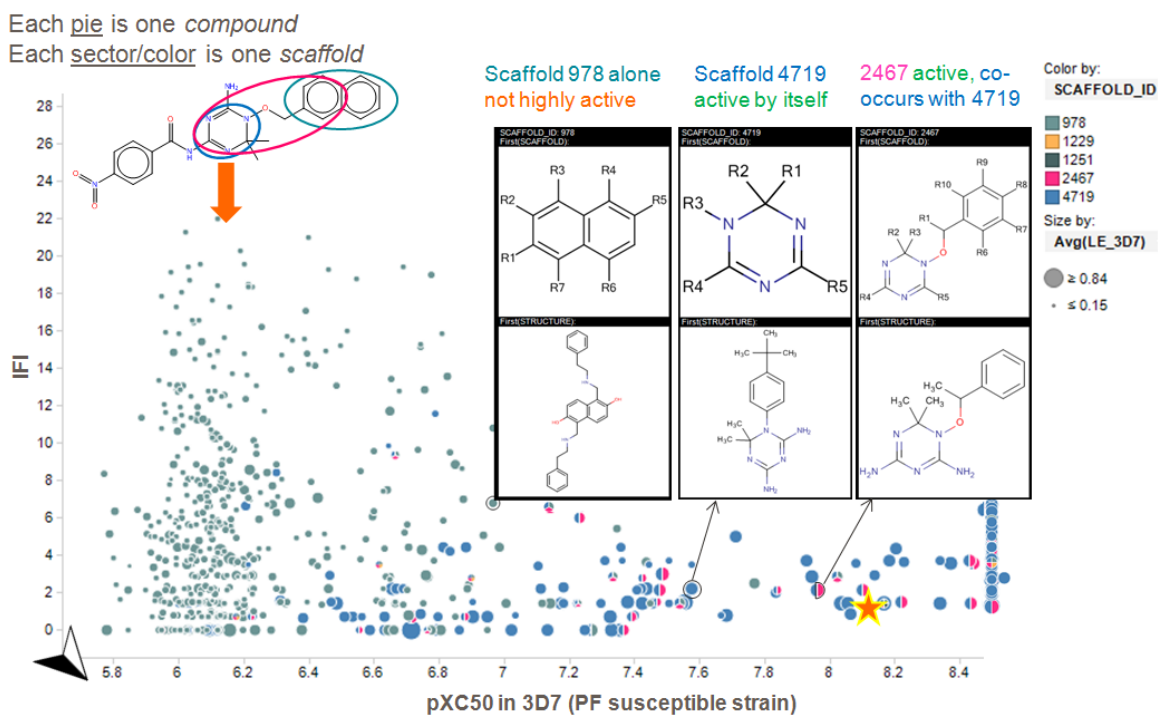


Figure 8: Related Molecules scaffold pies visualization for Molecule 1 (CID: 536182). Each pie here is one related molecule, and each pie sector and color is a scaffold that it shares with the parent molecule. The star symbol is added to show the location of the parent molecule in this plot, and the compass device at the origin shows the direction of favorable properties (in this case towards the +X and -Y axes). Insights derived from the plot are highlighted in the figure and also discussed in the text.

scaffold is observed to always occur where the dihydrotriazine-phenethyl-ether (#2467, pink) one does, implying the substructure relationship between them visually even if one did not know it beforehand. Scaffold #4719 also exists and is active without #2467, implying that the phenethyl ether can be substituted and the dihydrotriazine may be sufficient for activity by itself.

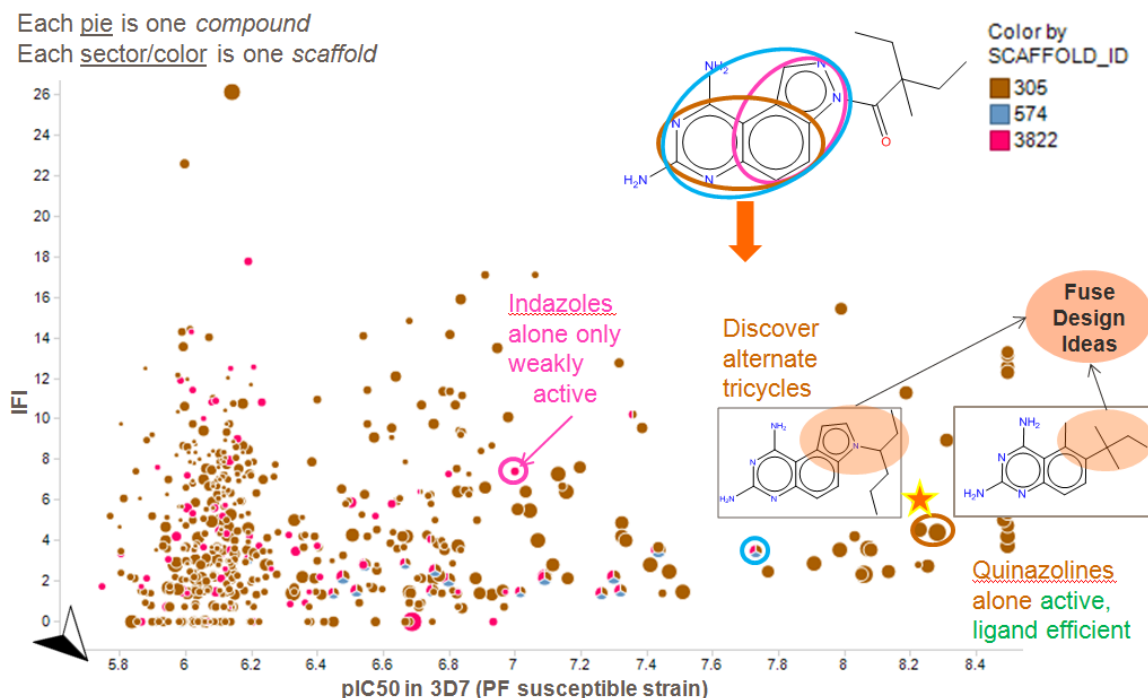


Figure 9: Related Molecules scaffold pies visualization for Molecule 2 (CID: 533945). Each pie here is one related molecule, and each pie sector and color is a scaffold that it shares with the parent molecule. The star symbol is added to show the location of the parent molecule in this plot, and the compass device at the origin shows the direction of favorable properties (in this case towards the +X and -Y axes). Insights derived from the plot are highlighted in the figure and also discussed in the text.

Molecule 2 in Figure 9 is another case where we might want to optimize the physical and chemical properties of the molecule without sacrificing activity or increasing promiscuity. Since solubility has been shown to go down with number of aromatic rings independent of lipophilicity,³⁶ walks that remove one or more of the three fused rings might be productive. By exploring the Related Molecules, we observe the SAR for three scaffolds: quinazolines (#305, brown), indazoloquinazolines (#574, blue) and indazoles (#3822, pink). We observe

from the plot a few more molecules containing all three scaffolds (tricolored pies, i.e., exact analogs of the parent molecule); all of these are less active than the parent. The indazoles when they occur alone (pink circles) are far less active than the parent, suggesting they do not contribute significantly to activity and may be substituted. Lastly the quinazolines (brown) include several analogs that are more active and also less promiscuous than the parent. Drilling down into these structures, we observe several that contain only two aromatic rings (i.e., no more are either fused or attached); these provide novel, active and ligand-efficient templates on which to build new analogs with enhanced solubility or other properties. Suggestions for which analogs to make can often be obtained by examining the SAR – for example, disparate aliphatic and aromatic analogs at two adjacent positions on the quinazoline phenyl are active, which suggests hybridizing them or designing further analogs substituted at these positions.

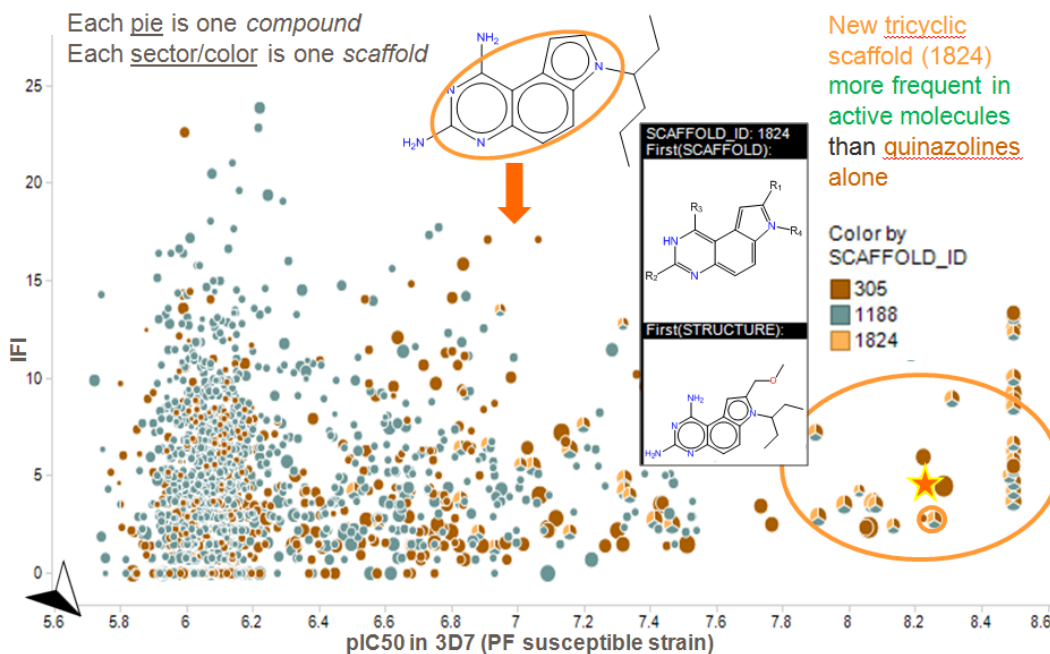


Figure 10: Iterative Related Molecules scaffold pies visualization for Molecule 3 (CID: 541531), a scaffold hop from Molecule 2 shown in Figure 9. Each pie here is one related molecule, and each pie sector and color is a scaffold that it shares with the parent molecule. The star symbol is added to show the location of the parent molecule in this plot, and the compass device at the origin shows the direction of favorable properties (in this case towards the +X and -Y axes). Insights derived from the plot are highlighted in the figure and also discussed in the text.

Another intriguing result is seen by observing a new tricyclic series that shares the quinazoline but adds an indole instead of indazole as the third fused ring. This alternative tricyclic template, while it may not confer solubility advantages, opens up a new area of chemical space. By iteratively seeking the Related Molecules for this new hit as shown in Figure 10, we observed that most of the active quinazoline analogs have this new tricyclic scaffold (#1824, tan wedges in tricolored pies) and relatively few contain only quinazolines (#305, brown circles). Also, indoles by themselves (#1188, blue circles) do not much better than indazoles, so this is a new synergistic effect discovered by scaffold walking from the original hit.

Qualitative Comparison of Scaffold-Generation Methods and Clustering

Complete-Linkage Clustering: As shown in Figure 11, the defining feature of a partitioning clustering is that every molecule maps to one and only one cluster. Thus if a chemotype is broken up among two or more clusters, using the cluster ID to map Related Molecules can retrieve only neighbors from the same cluster, ignoring the other cluster. This is not ideal for purposes of the visualization and navigation method presented here, as arbitrary neighbors would be excluded depending on how the clustering is defined. Thus we do not advocate the use of clustering, unless it is a fuzzy clustering where all meaningful class memberships a molecule might have are considered.

NCATS R-group tool: As opposed to the clustering method, if any two molecules share a common substructure that meets the standards required of a scaffold by the NCATS method (e.g., being bordered by rings on each end), then those molecules will be found to contain that shared substructure as a scaffold and their activities will be used to compute aggregate properties for it.

Other Scaffold Generation Methods: As shown earlier, even though other scaffold generation methods (GSK frameworks and Scaffold Network Generator) differed in their

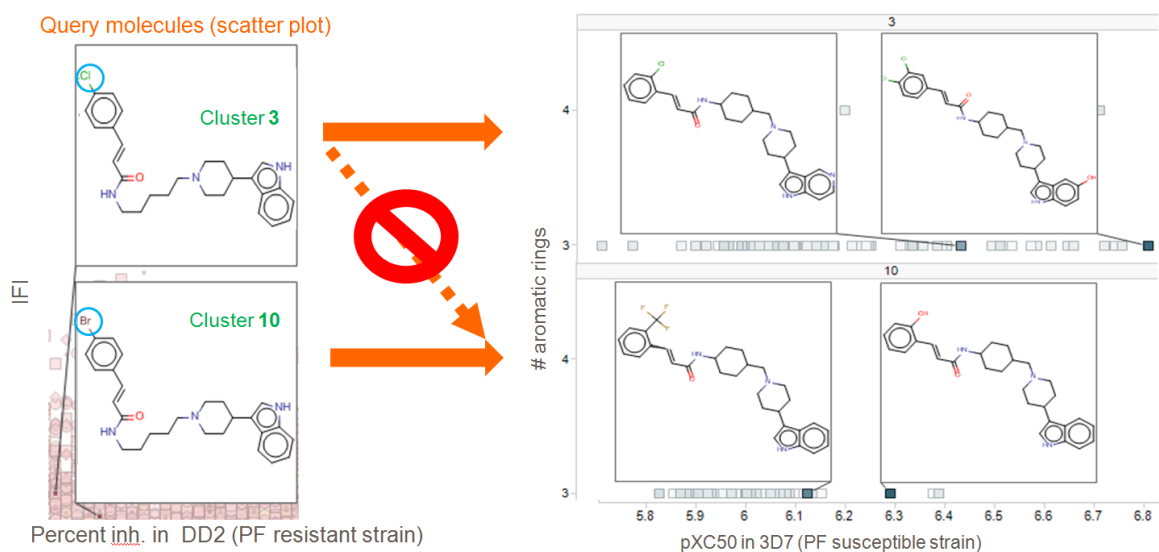


Figure 11: Illustrating one problem with clustering: bifurcation of related molecules. When two molecules of the same chemotype differing by a halogen are split across Complete Linkage Clusters, searches of cluster neighbors for one molecule do not find its analogs in the other cluster, i.e., the two related clusters are not linked.

implementation details and produced different numbers of scaffolds for the same molecule, they were roughly equivalent in a qualitative sense with regard to the insights obtained. Due to substantial overlap between sets of scaffolds, ring systems responsible for activity of a molecule were generally revealed by any of the methods. For example, the insights mentioned in Section were more or less consistent across the three methods. However, there were cases where the Frameworks revealed negative information about a fragment being not important for activity that is also useful for a drug discovery scientist. For example, in Figure 12 a substructure is highlighted that is on the aggregate inactive and could be removed or substituted. This insight is not available from the other methods since they don't define or find that fragment as a scaffold.

Using the Scaffold Network to visualize Related Molecules, one is able to deconvolute scaffolds with different numbers of rings (which serves as the level in the hierarchy) by trellising on it, and thus gather together substructures of comparable size. As shown in Figure 13, with the same tricyclic molecule as in Figure 9, we use Scaffold Networks to solve a selectivity issue. Members of the original scaffold, shown in light brown, tend to

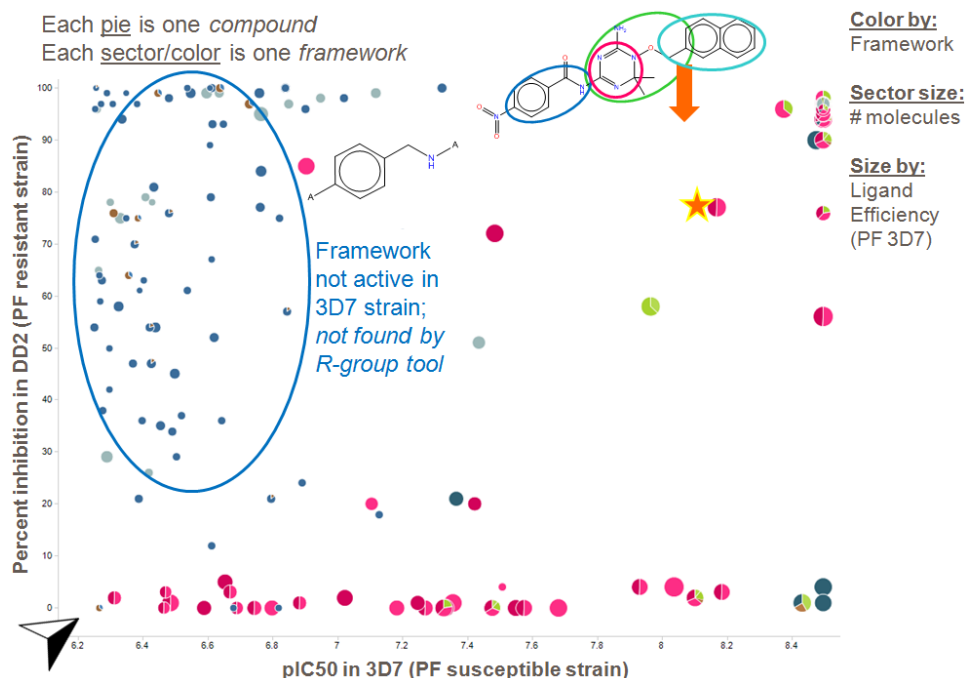


Figure 12: Using GSK Frameworks with the Scaffold Pies visualization. One framework is highlighted that has no equivalent in the NCATS scaffolds, but is shown to reduce activity as related molecules containing it are less active than the parent molecule. The star symbol shows the location of the parent molecule in this Related Molecules plot, and the compass device at the origin shows the direction of favorable properties (+X and +Y axes).

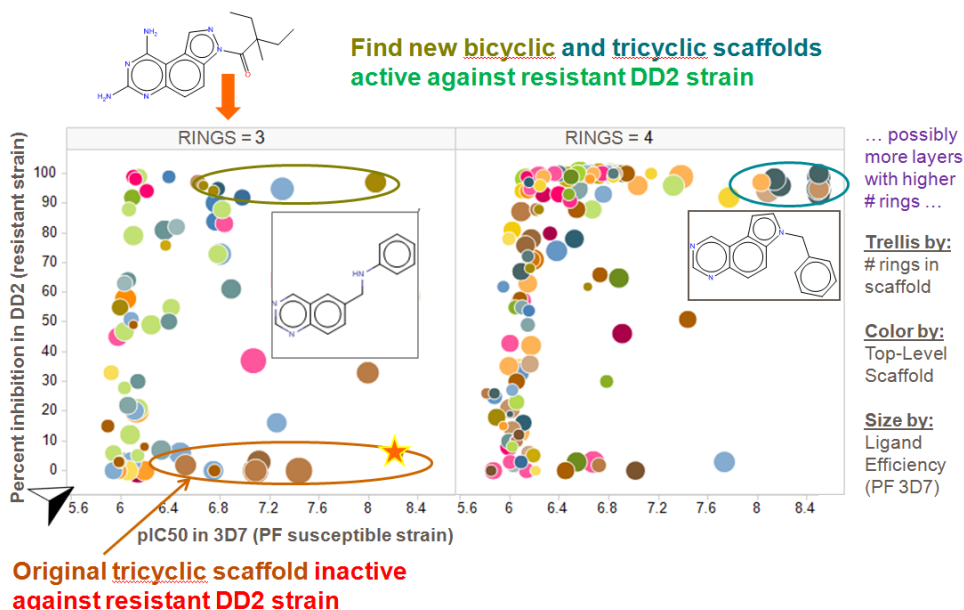


Figure 13: Using SNG to find Related Molecules a few hops away in the Scaffold Network that have a desirable property, in this case activity against the resistant DD2 strain of *P. falc.* The star symbol shows the location of the parent properties in this plot, and the compass device at the origin shows the direction of favorable properties (+X and +Y axes).

have some activity against the susceptible 3D7 strain of *P. falciparum* but be inactive against the multidrug-resistant DD7 strain. Using the scaffold network to go one level down (to molecules with two rings) and then two levels up (up to 5 rings) we obtain many new molecules that are a few hops away from the top-level tricyclic scaffold while still sharing some aspect of it. Looking at where these new Related Molecules are placed, we mark one scaffold with 3 rings that has a quinazoline linked to a phenyl, and one with 4 rings that contains an indoloquinazoline (also discovered by scaffold walking with the NCATS R-group tool as described above) linked to a phenyl. These two scaffolds are highlighted since their members consistently have high activity against the resistant DD2 strain, and thus this would be a desirable scaffold hop (or walk) towards chemical space that is more useful in overcoming resistance from the malarial parasite.

To summarize, all three multiple-scaffold decomposition methods considered in this study, i.e., NCATS R-group Tool, GSK Frameworks and Scaffold Network Generator give comparable insights when exploring the TCAMS dataset, with some differences stemming from individual substructures that are considered shared scaffolds or not by the individual methods. We now explore these overlaps, similarities and differences in the aggregate using the statistical methods described earlier in Section .

Statistical Comparison of Scaffold-Generation Methods

Recall the concepts of structure group and common proportion defined in Section . Let us now apply these to analyze and compare the scaffold generation methods GSK Frameworks (A) and NCATS R-group tool (B). As an illustration, Figure 14 (a)-(c) shows the structure group of the compound C with PubChem ID 536182.

Comparing the GSK Frameworks and NCATS R-Group Tool for the TCAMS dataset using the above mentioned statistical metrics, we show the aggregate statistics over the entire dataset in Figure 15.

We compare the Common Proportion, Proportion of Information Unique (PIU) and

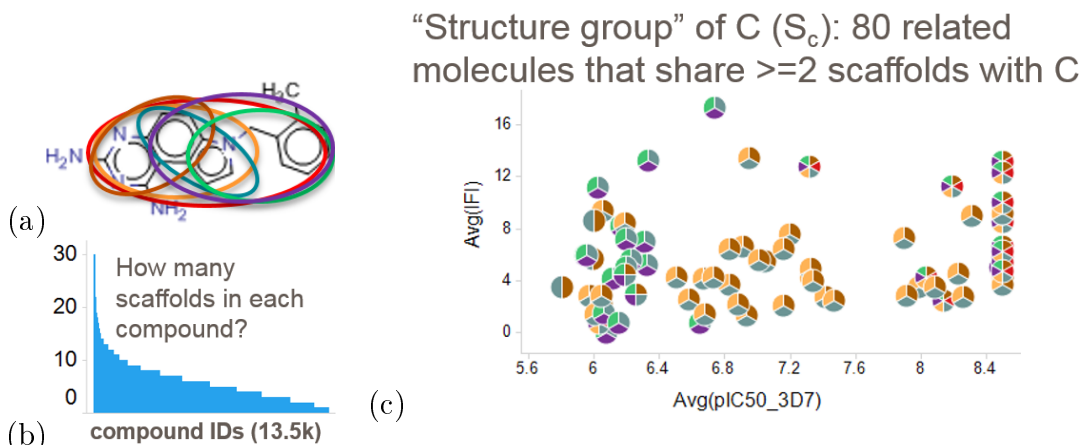


Figure 14: (a) Compound C , chosen here as PubChem ID 536182 from previous figures, has 6 fragments derived using the NCATS R-group tool. (b) Distribution of number of fragments, out of nearly 6000 total, found in each molecule. (c) Scaffold pie plot showing the structure group of C , restricted here to only compounds that share two or more substructures with C . This ensures that all compounds sharing just a single heteroaromatic ring are not in the same group, as chemists expect, and also reduces the group size – 80 related molecules for this particular compound C .

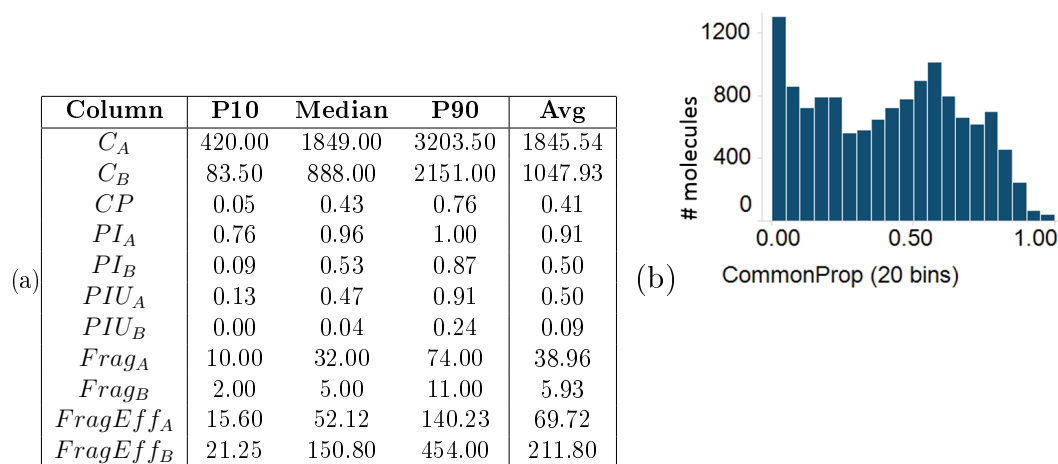


Figure 15: Statistics computed for 10th/90th percentile, median and average value for structure group size (C_A/C_B), common proportion, proportional information unique to A and B, number of fragments in and fragment efficiency of method A (GSK frameworks) and B (NCATS R-group tool).

Fragment Efficiency (FragEff) statistics for the GSK Frameworks (method “A”) and the NCATS R-group tool (method “B”) for all the 13.5k compounds in TCAMS in Figure 16. This figure has PIU of methods *A* and *B* on the axes, is sized by ratio of Fragment Efficiencies for all 13.5k molecules.

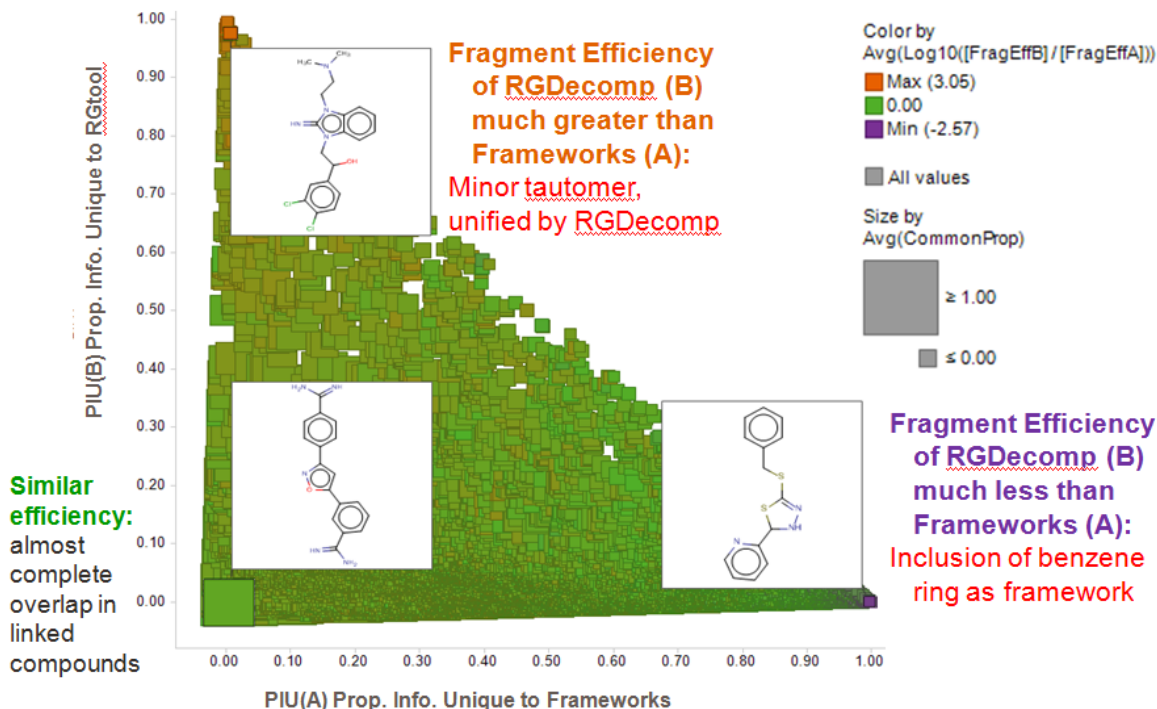


Figure 16: Comparison of the Common Proportion, PIU and FragEff statistics (described in the text) between GSK Frameworks (method “A”) and NCATS R-group Tool (“B”) for all molecules in TCAMS. PIU of A and B are on the X and Y axes, and the points are colored by the log ratio of FragEff(B) to FragEff(A) and sizes by Common Proportion.

We can make a few observations from this data and plot:

1. The two methods allow one to access different sets of molecules starting from any molecule in TCAMS - the average overlap in their coverage is 40%.
2. On the average, one can link to about twice as many molecules with the GSK frameworks; however, this is because on the average there are 6 times more frameworks than NCATS scaffolds, so the fragment efficiency is actually 3 times greater for NCATS scaffolds. One could also argue that many of the framework-only links (e.g., variously decorated benzene rings) are not useful.

3. The outliers are interesting. At one end, compounds in a rare tautomer are unified with the dominant one by the NCATS tool (high fragment efficiency), but left as singletons by the frameworks (low fragment efficiency). And compounds whose only link with other molecules would be a benzene ring or similar low complexity scaffold remain singletons with the R-group tool (lower fragment efficiency).

Statistical Basis of Structure-Activity Relationships (SAR)

We have also explored the Common Proportion measure to get at the overlap between structure and activity, i.e., to investigate the statistical basis of SAR based on overlapping scaffolds. To do this, we define an Activity Group of compound C , C_{act} as a set of compounds with activity bordering that of C (in this case pIC50 in the 3D7 antimalarial assay). The activity group of each compound C is defined to be the same size as the structure group, denoted C_A or C_B above, and generalized here as C_{str} . This is done arbitrarily for ease of statistical calculations, being cognizant that it may sometimes lead to issues such as breaking a large list of compounds with the same activity arbitrarily, especially when picking a small list of activity neighbors for compounds with a small structure group.

The structure-activity common proportion, $CP_{str,act}(C)$ then measures the overlap between the structure neighbors of C , i.e., compounds sharing any scaffold with C , and its activity neighbors, i.e., compounds in a similar activity range.

$$CP_{str,act}(C) = \|C_{str} \cap C_{act}\| / \|C_{str} \cup C_{act}\| \quad (6)$$

For comparing groups of different sizes we use the normalized Common Proportion, dividing by the expected overlap of the same activity group with a structure group determined purely by chance. With the structure group being the same size as the activity group, this is the probability that if we pick $\|C_{act} - 1\|$ compounds at random they will lie in C_{act} , i.e., $(\|C_{act} - 1\|) / (N - 1)$. The subtraction by 1 accounts for the fact that the compound

itself always lies in its own structure and activity neighborhoods. So then:

$$NormCP_{str,act}(C) = CP_{str,act}(C)/(ExpectedCP for Random Str) \quad (7)$$

We computed this NormCP measure for all compounds, which fit a normal distribution centered at 1, implying that for all compounds considered as an aggregate, structural similarity does not imply activity similarity. However, the top 200 most active compounds, which are activity outliers, are also outliers in this normal distribution of NormCP as shown in Figure 17(a).

Among these compounds there are some that have over 40 times as much overlap between structure and activity than would be expected by chance, shown on the right of Figure 17(b) and in Figure 17(c). This implies a strong confidence in the activity being genuine, and the series being worthy of making or measuring the activity of further analogs.

On the other hand, there are also some compounds, highlighted on the left side of Figure 17(b) and in Figure 17(d-e), that though they are among the top 200 by activity, have NormCP much less than 1, i.e., much less overlap between structure and activity than would be expected by chance. This would imply that structural neighbors of these compounds are largely less active than the original compound, no matter which of the overlapping scaffolds within the molecule we use to define those structural neighbors! This gives us a way to quantify flagpoles or false positive hits, which may not be worth following up.

Discussion

While threshold-based hit selection is a prevalent approach in the analysis of high throughput screening datasets, it is simplistic and ignores the extra information encoded in chemical structure. Thus scaffold based analysis of high throughput screening datasets represents a truly data-driven approach to hit triage that attempts to make use of all the data collected from a high throughput screen. Ranking scaffolds is a key step in prioritizing hits in a

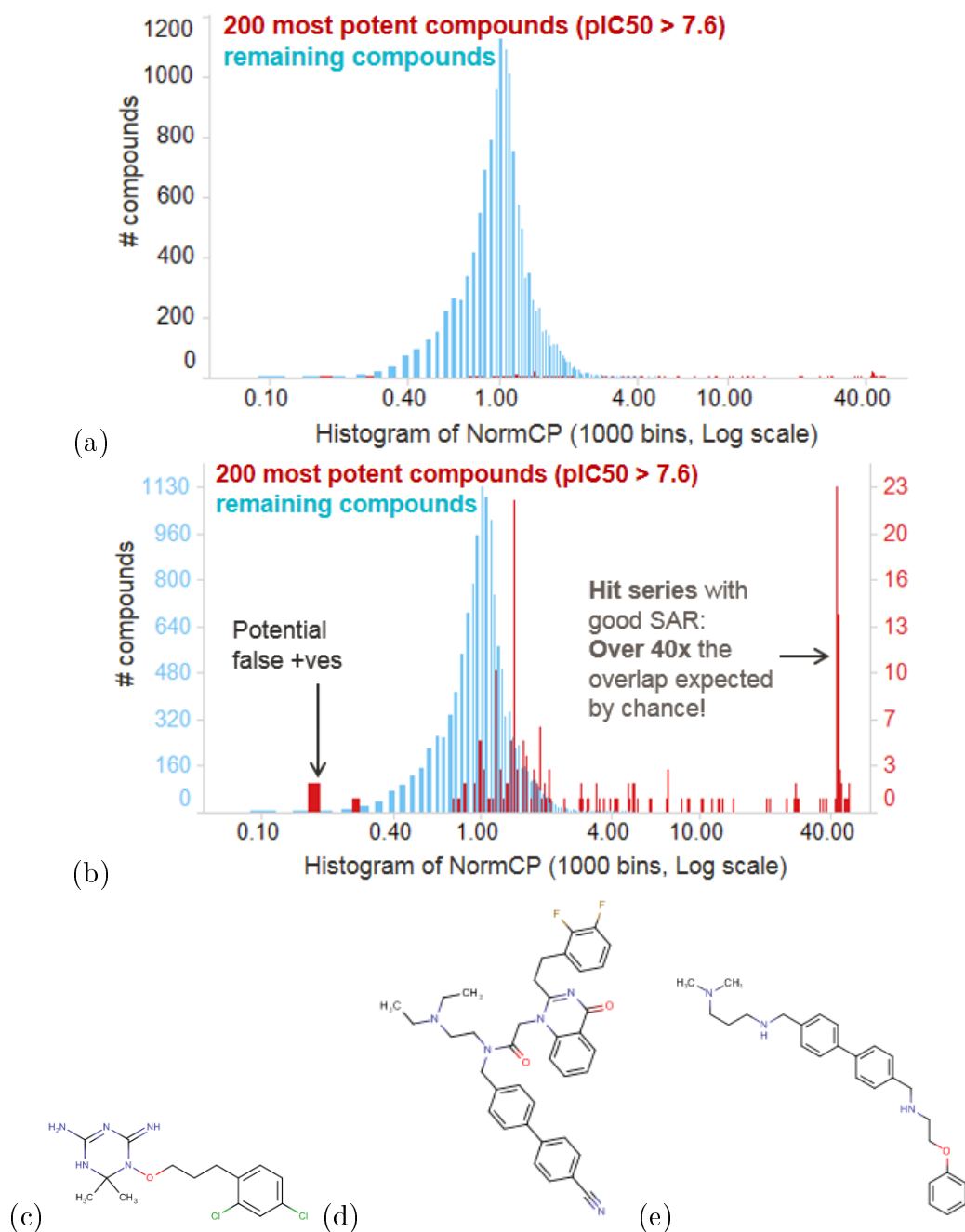


Figure 17: (a) Normalized common proportion scores between structure (data-driven frameworks) and activity ($pIC_{50_{D7}}$) for all 13.5k molecules in TCAMS. The histogram has 1000 bins and a log scale on the X-axis. The top 200 most active compounds ($pIC_{50_{D7}} > 7.6$) are marked in red, and occupy many of the outlier bars with high NormCP > 10 . (b) Making the distribution of the top 200 active compounds clearer by giving them a separate scale, at the RHS. (c) One of the 23 compounds with NormCP = 42.5, PubChem CID 524739. (d)–(e) two active compounds with low NormCP in the 0.16–0.17 range: (d) CID 542941 and (e) CID 531249. For both compounds, they alone are highly active among a structure group of 96 compounds, raising the probability that these compounds are flagpoles and not worth following up as hits.

scaffold-based approach, and while there are many ways to generate a ranking, it is not obvious which method is optimal (or whether there is even a single, optimal method).

In this study we have presented some alternate approaches to scaffold prioritization that make use of aggregate statistics based on overlapping scaffolds, with the goal of provide a quantitative basis for the comparison of different scaffold-based analysis schemes.

We observe that this approach, applied to the kinase "X" dataset can be a powerful tool to identify promising hits. While a simple procedure, the use of the Spotfire workflow allows interactive drill down from aggregate properties to individual compounds. In essence, the workflow described here enables decisions on individual compounds, but using the aggregated data as a filter. Another advantage of the workflow described here is that it supports the inclusion of "virtual datasets", where there is no measured activity, as highlighted in the data fusion use case. Inclusion of such datasets can be useful as they provide an opportunity to directly highlight untested regions of chemical space. When multiple datasets, with measured activities, are included in the data fusion, it provides the opportunity to increase confidence in noisy data by merging data for scaffolds across the datasets.

Compared to partitioning-based clustering methods (e.g., complete linkage clustering), the overlapping scaffold approach described here avoids the phenomenon of similar molecules being arbitrary assigned to exclusive clusters. Instead, using overlapping scaffolds, will consider such molecules as a single group.

Given the different ways to generate scaffolds and the compute overlapping scaffolds, a quantitative approach to characterizing differences in these approaches is necessary. The use of common proportion (CP), fragment efficiency (FragEff) and PIU places such comparisons on a sound statistical basis, allowing for an objective comparison of fragmentation methods for given screen. As mentioned earlier they also extend the applicability of methods to compare the output of different partitioning clustering methods such as Torres et al., allowing them to be used for non-overlapping fuzzy clusters. Furthermore quantifying structure-activity overlap using NormCP is a novel contribution, though similar in spirit and purpose

to local hit rate calculations that have been proposed for HTS triage,³⁷ with a comprehensive structural neighbor metric based on overlapping scaffolds.

In summary, the combination of anecdotal and statistical methods to compare scaffold schemes and the resultant analysis of HTS datasets highlights the fact that no single fuzzy clustering method is optimal, and the most appropriate approach should be selected based on the types of analyses described here. It is a given that chemical intuition plays a role when examining scaffolds, yet such intuition can lead to biased selections of scaffolds and subsequently of leads. The data driven approach coupled with intuitive visualizations, as described in this study, provides a way to combat such biases.

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Supporting Information Available

Supplementary material is available online for this article.

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