ABSTRACT

Summary: The chemical structures of biomolecules, whether naturally occurring or synthetic, are composed of functionally important building blocks. Given a set of small molecules—for example, those known to bind a particular protein—computationally decomposing them into chemically meaningful fragments can help elucidate their functional properties, and may be useful for designing novel compounds with similar properties. Here we introduce molBLOCKS, a suite of programs for breaking down sets of small molecules into fragments according to a predefined set of chemical rules, clustering the resulting fragments, and uncovering statistically enriched fragments. Among other applications, our software should be a great aid in large-scale chemical analysis of ligands binding specific targets of interest.

Availability and implementation: molBLOCKS is available as GPL C++ source code at http://compbio.cs.princeton.edu/molblocks.

Contact: mona@cs.princeton.edu

Supplementary information: Supplementary data are available at Bioinformatics online.

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1 INTRODUCTION

Endogenous small molecules are synthesized in the cell in a modular fashion, using building blocks or fragments that are often conserved across organisms (Muto et al., 2007). Fragment-based drug discovery has also emerged as an important paradigm to navigate the diversity of the chemical landscape and to profile protein druggability (Hajduk and Greer, 2007). Further, it has been shown that the toxicity of certain drugs can be explained by the presence in their structure of fragments that are shared by toxic compounds (Ahmed et al., 2009). Although many programs are available to assemble small molecules from fragments (Schneider and Baringhaus, 2013), the reverse problem of breaking down small molecules and analyzing the corresponding fragment sets has been studied less extensively. An implementation of the RECAP algorithm (Lewell et al., 1998) to fragment small molecules can be found in a commercial program (fragmenter, www.chemaxon.com), and is available in the RDKit library (http://www.rdkit.org), which also implements the BRICS fragmentation algorithm (Degen et al., 2008). However, given a diverse set of small molecules that share a property of interest, there is no automated tool to identify statistically enriched fragments that might explain their activity.

Here we introduce the molBLOCKS suite, which allows users to break down small molecules into chemically meaningful fragments and analyze the resulting fragment distribution (Fig. 1). The software consists of two command-line programs: fragment and analyze. The fragment program reads user-defined rules to specify the bonds to break or uses default sets of rules [RECAP (Lewell et al., 1998), CCQ (www.chemaxon.com), and BRICS (Degen et al., 2008)]. Then, the program applies these rules to fragment the molecules, and generates all fragments with a number of heavy atoms above a minimum size defined by the user.

The analyze program collects statistics on the frequency with which each fragment occurs, clusters fragments using a user-defined similarity threshold based on a fingerprint representation (O’Boyle et al., 2011) of the fragments and selects a representative fragment for each cluster. This program can also perform enrichment analysis at the level of either fragments or clusters.

A typical scenario where fragment and enrichment analyses can be applied is when dealing with a library of small molecules, a subset of which has a specific property of interest. In these cases, molBLOCKS can be used to fragment the whole library and determine which (if any) fragments are significantly enriched in the set with the property of interest. Fragmentation and enrichment analysis of small molecules may also be useful in analyzing proteins. For example, ligands bound by proteins that share a common property, such as a specific function, can be analyzed in this manner. Such an approach would provide a complement to the functional enrichment analyses that are routinely performed with Gene Ontology terms (Huang da et al., 2009).

Extensive fragmentation of the entire DrugBank (Wishart et al., 2006) collection of 6460 small molecules with the default rules took 53 s on an iMac with a 2.66 GHz processor. A user’s guide with implementation details and more tests is provided with the suite.

2 METHODS

2.1 fragment

Small molecules and bond-breaking rules are specified with SMILES (Weininger, 1988) and SMARTS (Daylight Inc.) notation, respectively. The open-source Open Babel C API (O’Boyle et al., 2011) is used to
The `fragment` program takes as input a set of small molecules and user-defined rules that specify the bonds to break, and then applies these rules to fragment the molecules. As an optional second step, carried out by the `analyze` program, the user can cluster the fragments and/or determine whether the frequency of any of the fragments is enriched as compared with a background set of fragments.

Without extensive fragmentation, the program returns only one possible fragment - the largest one. For a given threshold of similarity, a graph is created where there is a directed edge between two nodes if both bonds can be cut; the Tanimoto coefficient between the fingerprint representations is used to compute their fragment similarity. Subsequently, we identify the enriched fragments in a background dataset of drugs in KEGG with the `analyze` program. The `analyze` program returns both uncorrected P-values and FDRs obtained with the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995) to handle multiple hypothesis testing.

### 3 USAGE

As an example of how to use the `molBLOCKS` suite, we fragmented a set of antineoplastic drugs extracted from KEGG (Kanehisa et al., 2012) with the following command:

```
fragment -i antineoplastic.smi -r RECAP.txt -n 4 -e antineoplastic.frag –e
```

where `antineoplastic.smi` is a text file containing the small molecules in SMILES format to fragment. The `RECAP.txt` file contains a definition of the cleavable bonds, encoded as SMARTS patterns. The `-e` flag specifies extensive fragmentation, and the `-n` parameter controls the minimum size of a fragment, defined as the total number of heavy atoms. The `antineoplastic.frag` file contains the output of the fragmentation.

Subsequently, we identified the enriched fragments in a background dataset of drugs in KEGG with the `analyze` program:

```
analyze -i antineoplastic.frag -c 0.8 -e background.frag –o distr.txt
```

With the optional `-c` parameter, `analyze` clusters the fragments at the specified Tanimoto coefficient. The optional `-e` parameter specifies the background set for enrichment analysis; this set must contain the fragments in the input set for the results to be meaningful. Figure 2 shows an example of an enriched fragment and its parent molecules in the antineoplastic set. See the Supplementary Materials for further details.
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