Updated Prediction of Aggregators and Assay Interfering Substructures in Food Compounds

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ABSTRACT

Positive outcomes in biochemical and biological assays of food compounds may appear due to the well-described capacity of some compounds to form colloidal aggregates that adsorb proteins, resulting in their denaturation and loss of function. This phenomenon can lead to wrongly ascribing mechanisms of biological action for these compounds (false positives), as the effect is non-specific and promiscuous. Similar false positives can show up due to chemical (photo)reactivity, redox cycling, metal chelation, interferences with the assay technology, membrane disruption, etc., which are more frequently observed when the tested molecule has some definite interfering substructures. Although discarding false positives can be achieved experimentally, it would be very useful to have in advance a prognostic value for possible aggregation and/or interference, based only in the chemical structure of the compound tested, in order to be aware of possible issues, help in prioritization of compounds to test, design of appropriate assays, etc. Previously, we applied cheminformatic tools derived from the drug discovery field to identify putative aggregators and interfering substructures in a database of food compounds, the FooDB, comprising 26457 molecules at that time. Here we provide an updated account of that analysis based on a current, much-expanded version of the FooDB, comprising a total of 70855 compounds. In addition, we also apply a novel machine learning model (the SCAM Detective) to predict aggregators with 46%-53% increased accuracies over previous models. In this way, we expect to provide the researchers in the mode of action of food compounds with a much improved, robust, and widened set of putative aggregators and interfering substructures of food compounds.
KEYWORDS: Food compounds, aggregators, interference filters, PAINS, assay

interference, promiscuous compounds, cheminformatics, SCAM Detective
INTRODUCTION

There is currently a great research effort in the identification of the biological mechanisms of action of food compounds from a molecular point of view, in order to understand the beneficial or harmful effects of foods on human health, as well as finding novel nutraceuticals and scaffolds for drug design.\textsuperscript{1-9} For that aim, biochemical and/or biological (cellular) assays directed towards different biological targets (typically proteins) are being conducted, so that specific macromolecule-food compound interactions can be identified. However, these assays are subject to compound-related artifacts. For example, compound aggregation is a well-described phenomenon that yields artifacts in biochemical and biological assays.\textsuperscript{10-14} Some compounds, due to low water solubility, when tested at concentrations above a critical aggregation concentration (CAC),\textsuperscript{15} form colloidal aggregates that adsorb biomacromolecules nonspecifically and alter their activities, in most cases inhibiting them through denaturation,\textsuperscript{16} although in some cases activating them.\textsuperscript{17} This effect can translate into misled interpretations of the biological mechanism of action of compounds, as it is wrongly ascribed to the target used in the assay while the aggregation is nonspecific. Aggregation is very dependent on the assay conditions (pH, buffer composition, testing concentration) and structure of the compound, rather than on the assay technology and target, and can be alleviated to some extent by the addition of nonionic detergents in the assay medium.

An alternative source of false positives in assays is the presence of substructures that make the molecule interact promiscuously with many targets, through mechanisms like (photo)chemical reactivity, large hydrophobicity, redox cycling, metal chelation, etc.; or
that provide it with some interfering properties with the assay technology (absorption/emission at reading wavelengths, membrane disruption, singlet-oxygen quenching or production, etc.).\textsuperscript{18-26}

There is a soaring concern for the presence of increasing numbers of false positives in the scientific literature and the derived databases of bioactivities due to aggregation and/or interfering substructures, as it could severely hurt knowledge extraction, decision making, and lead to the waste of resources and time.\textsuperscript{27,28} As a matter of fact, the American Chemical Society has provided specific guidelines\textsuperscript{28} to follow when submitting manuscripts reporting biological or biochemical activities of compounds with putative aggregation and/or interfering substructures, such as performing additional orthogonal assays (with the same target but different technology) and/or counter-assays (with different target but the same technology), adding non-ionic detergents in the assay buffer, and reviewing reported activities for the same molecules in the literature.

In a previous work,\textsuperscript{29} we performed an systematic analysis of a large database of food compounds, the FooDB,\textsuperscript{30} aiming at identifying there both putative aggregators and interfering substructures. This effort would be useful for the scientific community aiming at deciphering the biological mechanisms of action of food compounds, as it would allow to point out possible issues with reported activities, guide in the prioritization of compounds to test, and help in the assay technology selection and design. We used for the analysis well-established and publicly available cheminformatic tools,\textsuperscript{12,19,20,22} derived from the statistical and machine learning analysis of a large number of structure-activity datasets from both the high-throughput screening and
medicinal chemistry fields: to identify putative aggregators, we employed the
Aggregator Advisor model,\textsuperscript{12} while to find putative interfering substructures, we used
three standard libraries of substructural filters derived through data mining efforts: the
Pan-Assay INterference compoundS ("PAINS") set,\textsuperscript{22} that of former-GlaxoWellcome
(here called "Glaxo"),\textsuperscript{19} and another from Pfizer ("LINT").\textsuperscript{20}

Since 2020, the FooDB size has increased considerably, from about 26000 compounds
at the time when the previous paper was published, to about 71000 to date. This
suggested the need for an update of this analysis, as the number of compounds in the
current version more than duplicates that of the previous one, leaving a lot of molecules
without analysis for possible aggregation and/or interfering behavior. In addition, we
took the opportunity to use more advanced approaches to predict aggregation. The
Aggregator Advisor uses a simple and conservative approach for prediction, as it relies
on a database of \textasciitilde12000 known aggregators, and only marks a new compound as
aggregator if its Tanimoto similarity to one or more know aggregators is \textasciitilde0.85 and its
logP \textasciitilde3. Thus, this approach is restricted to a chemical space very close to the known
aggregators, not being able to extrapolate to molecules outside this narrow space. It
basically suggests putative aggregators with confidence, but potentially misses the vast
majority of the chemical space, and contains no information about non-aggregators. On
the contrary, a recent model based on machine learning, the so-called SCAM
Detective,\textsuperscript{31} is based on balanced datasets comprising tens of thousands of compounds
for both the aggregator and non-aggregator classes, in two assays, each repeated with
and without detergent, and at different concentrations of compound (here SCAM stands
for "small, colloidally aggregating molecules"). The use of a much larger and balanced
dataset, together with a Random Forest as predictive model, as well as Extended-Connectivity Fingerprints of diameter 6 (ECFP6) to represent molecular structures, allows to increase the sensitivity and specificity of the predictions, resulting in improvements of total accuracy from 46% to 53% over previous models (e.g. the Aggregator Advisor), and to make it applicable to the whole chemical space. In addition, the SCAM Detective allows to generate probability maps for aggregation, that highlight regions in the molecule with high and low propensity for aggregation and help in the interpretation of the predictions in molecular terms.

Thus, in this work we attempt to provide an updated and more robust analysis of the putative aggregators and interference substructures in the current FooDB, comprising near 71000 molecules. We expect this work will be valuable for the experimentalist testing food compounds in different biological targets, in order to reduce the presence of false positive reports in the literature for these molecules. In turn, this will help globally to gain a better understanding of their true biological mechanisms of action and structure-activity relationships.
MATERIALS AND METHODS

All the analyses were performed in Python 3.9 and with the RDKit cheminformatic toolkit,\textsuperscript{32} version 2021.03.3. The FooDB,\textsuperscript{30} comprising a total of 70855 molecules (including all detected and quantified, detected but not quantified, expected but not quantified, and predicted) were kindly provided by Dr. Wishart group in SDF format. Structures were checked in a first step, and then standardized and solvent- and counterion-stripped (in the case complex molecules containing counterions and/or solvent molecules), to yield the corresponding parent compound, by using the ChEMBL Structure Pipeline.\textsuperscript{33} A few compounds could not be processed by RDKit. Some compounds raising the Pipeline’s exclusion flag (i.e. with transition metals, or > 7 of boron atoms, that cannot be properly standardized) or with penalty scores > 5 (due to severe structural inconsistencies) were discarded (molecules with penalty score = 5 were kept as these corresponded to stereo mismatches between InChi vs RDKit vs Mol representations of the molecules, with no impact in the analysis of aggregators or interference substructures). After that, duplicates were removed based on the InChiKey, resulting in a total of 69502 unique molecules. The application of this recently described structure normalization procedure changed slightly the statistics for the molecules analyzed in our previous work and reported therein,\textsuperscript{29} that were now reanalyzed as they were a subset of the new FooDB, but we think this provides a more robust analysis of aggregators and interference substructures, without changing the former conclusions. In some cases, for comparison purposes a list of drugs was used. This was obtained from the DrugBank,\textsuperscript{34} using the small molecules in approved, not-withdrawn, and non-illicit
status as in our previous work, and following normalization procedure as with the FooDB. The resulting number of unique molecules was 2154.

For the SCAM Detective calculations to predict aggregators, the code provided by the authors was locally installed and adapted for batch calculations. For comparison purposes with our previous work, the aggregators were also predicted through the Aggregator Advisor method, implemented in a locally programmed script with RDKit as the Aggregator Advisor does not provide batch processing functionality. In this script, a molecule was assigned a “known” status if its Tanimoto similarity with a molecule in the list of 12642 Aggregator Advisor molecules was 1, a status of “probable” if its largest Tanimoto similarity with any molecule in the list of aggregators was ≥ 0.85 and its logP > 3, and a status of “none” for the rest of the molecules.

To test differences in distributions of numeric variables in different groups, the non-parametric Kruskal-Wallis one-way analysis was used, followed by Conover’s post-hoc test with p-values correction using Holm’s approach. To test differences in proportions for pairs of samples, Z-test for proportions were used.
Physicochemical and structural analysis of the molecules in the new FooDB

Prior to conducting our analysis of aggregators and interfering substructures, it seemed necessary a characterization of the molecules incorporated in the new release of the FooDB, in order to see the extent of overlap with the chemical space of the previous FooDB molecules. A very large subset of the additional molecules in the new FooDB corresponds to acylglycerols (in what follows abbreviated AG), namely compounds with a glycerol backbone and with at least one fatty acid esterified to it, although other types of molecules (non-acylglycerols or NAG) are present. Figure 1 displays different in-silico calculated physicochemical and structural properties for four groups of molecules, specifically the molecules in the previous FooDB (corresponding to the “FD(OLD)” label in the abscissa), new non-acylglycerol molecules in FooDB (likewise, “FD(NEW/NAG)”), new acylglycerol molecules in FooDB (“FD(NEW/AG)”), and DrugBank (“DB”) molecules. The descriptors calculated were: TPSA (topological polar surface area, TPSA), logP (LOGP), number of rotatable bonds (RB), number of hydrogen bond donors (HBD), number of hydrogen bond acceptors (HBA), molecular weight (MW), QED (Quantitative Estimation of Drug-likeness, QED\textsuperscript{35}), number of rings (NRING), and fraction of sp3 carbons (FSP3).

We can see in Figure 1 that the dispersion of distributions is quite different for NAG and AG molecules in the new FooDB molecules. The main descriptive statistics and test for differences (omnibus and post-hoc) in the distributions of all the four groups of molecules can be obtained in Supporting Information (Tables S1 and S2). While the NAG tend to have a widening of their distributions as compared to the old FooDB, the AG
have usually very narrow distributions, with TPSA, HBD, HBA and NRING having a null inter-quartile range, which is expected given the high structural redundancy within this group as they are all acyl esters of glycerol. Both NAG and AG display a trend towards more and larger LOGP, RB, HBA, MW and FSP3. This is especially clear for the AG as regarding LOGP, RB and FSP3, and expected given their highly aliphatic structure. On the other hand, both NAG and AG display a reduction in QED and NRING, again not unexpected for the AG molecules.

As regarding TPSA, NAG show a marked increase compared to the old FooDB, while AG show an slight decrease of it; in the case of the HBD, the AG molecules have no one, while NAG show a clear trend towards higher values.

On the other hand, Figure 2 displays the distributions of the 10 most frequent Bemis-Murcko\textsuperscript{36,37} scaffolds for FDB(OLD), FDB(NEW/NAG), and DB. AG molecules are not shown as any of them have scaffold (they are linear, branched molecules, and therefore have no Bemis-Murcko scaffolds, which are ring-based). Again, we observe quite different distributions for these sets, in spite the benzene ring being the most frequent scaffold in all of them. The percentage of molecules without scaffold is lowest in the drugs, but highest in the NAG (after AG, which are 100%). Following the benzene scaffold, the drugs show a variety of typical drug scaffolds; in decreasing order, two steroid scaffolds, pyridine, diphenylmethane, another steroid, etc. In turn, the old FooDB molecules have in common the presence of steroid scaffold, but the second most frequent one is cyclohexane, followed by tetrahydropyran, etc., while the NAG compounds display completely different scaffolds: e.g. dual aliphatic esters ending in furan rings, cyclopentamine, imidazole, etc.
In summary, we see that the new FooDB molecules, both AG and NAG, appear to occupy a different region of the chemical space (including physicochemical properties and structures), more separated to that of the drugs, as compared to the previous release of FooDB. This observation stresses the need for an updated analysis of aggregators and interference substructures in the FooDB.

Aggregators Analysis

In our previous work, the Aggregator Advisor method\textsuperscript{12} was used to predict putative aggregators and identify known aggregators. The Aggregator Advisor is based on a very simple approach, comprising the calculation of the Tanimoto similarity of the tested compound to a list of $\sim$12600 know aggregators (using topological fingerprints), and if the similarity is $>0.85$ to at least one of these compounds, and its logP is $>3$, it is assigned a “possible aggregator” status; in the rest of the cases no prediction is made (“unknown”, or “non-aggregator” status). Thus, it is a very conservative approach that is unable to give predictions of compounds lying outside the close structural space of the aggregators list, although it is useful as a fast and easy-to-implement way to identify close analogs to the known aggregators list with high risk of aggregation if lipophilic enough.

On the contrary, the SCAM Detective\textsuperscript{31} is a machine learning-based approach using random forests\textsuperscript{38} that is capable in principle to extrapolate to the whole structural chemical space. The training sets for the SCAM Detective were obtained from pairs of quantitative high-throughput screening (qHTS) campaigns in PubChem\textsuperscript{39} run with the same assay conditions but in the presence and absence of added detergent in the assay buffer. By comparing the dose-response curves in each pair for each compound, in the
presence and in the absence of detergent, it was possible to mark it as putative aggregator or non-aggregator. Models were derived for both AmpC β-lactamase and the cysteine protease cruzain, which are frequently used counter-assays to discard false positives in assays. The corresponding screens in PubChem were tested at different experimental conditions in terms both of assay buffer and dosing concentrations. This is an interesting feature as by comparing the predictions in both assays an approach to assess the robustness of the prediction is available.

In addition, in the development of the training set, a data-rebalancing approach was applied, so that in both training sets there were equal numbers of aggregators and non-aggregators. In this way, the SCAM Detective is able to predict both aggregators and non-aggregators, and thus have a balanced sensitivity and specificity (0.72 and 0.73, respectively for the AmpC β-lactamase model, and 0.71 and 0.69 for the cruzain model).

The SCAM Detective also provides a measure of the reliability of its predictions, which is based on the so-called applicability domain (AD) of the model, defined as

\[ D_{\text{cutoff}} = \langle D \rangle + 0.5S \]

where \( \langle D \rangle \) and S are the average and SD of all the Euclidian distances in the descriptor space used between each compound and its nearest neighbors in the training set. New compounds with a minimum distance to the molecules in the training set \( D > D_{\text{cutoff}} \) would be outside the AD of the model, meaning that the predictions for it would be in general less reliable.

On top of that, prediction of fragment contributions in the form of contour maps can be generated for the modeled molecules, aiding to provide an interpretation of the model.
prediction through the groups in the compound most responsible for the aggregating or non-aggregating behavior.

By first applying the Aggregator Advisor method to the updated FooDB, we can identify a total of 92 known aggregators and 37 predicted aggregators. These concentrate mainly in the old FooDB database, since within the new compounds no one was a predicted compound, and only two were known aggregators. These were in the NAG group. By merging known and predicted aggregators, this leads to a 0.56% and a 0.004% of aggregator rate for the old and new FooDB molecules, respectively. These small numbers reflect the conservativeness of the Aggregator Advisor and that the novelFooDB molecules display even less overlap with the chemical space of the list of aggregators used by the method compared with the previous FooDB.

These numbers change dramatically upon application of the SCAM Detective. Table 1 displays the total number and percentages of predicted aggregators in both FooDB (and its different subsets) and DrugBank (for comparison purposes) for both the β-lactamase and cruzain models, as well as their intersection. The actual predictions for all the molecules in FooDB can be obtained in Supporting Information (Table S3). It can be seen that the aggregator rates for the SCAM Detective are much higher. For instance, the β-lactamase model predicts a 76.7% of aggregators in FooDB, that rises up to 95.39% for the AG subset. For the cruzain model, the aggregator rate is 40.82% for the whole FooDB, and 52.98% for the AG subset. In both models, the aggregator rates for the newFooDB molecules are significantly higher than for the old ones (43.17% and 19.95%, for β-lactamase and cruzain, respectively), both for NAG and AG, although especially for the later. This can be expected from the more lipophilic and flexible nature of these
molecules, with long aliphatic chains that makes them prone to aggregation in the AG.

As a way of confirmation, Figure 3 displays the predicted fragment contribution maps for FDB00135, a predicted non-aggregator in both models from the old FooDB, and FDB080642, a doubly-predicted aggregator and an AG compound. We can see that while FDB000135 shows green contours indicative of non-aggregation contribution together with some weakly concentrated magenta contours, FDB080642 displays highly concentrated and dark-magenta contours, a signature of high-aggregating contribution, especially in its three polymethylene chains.

On the other hand, the DrugBank display aggregator rates near the old FooDB molecules, slightly lower: 34.44% and 18.89% for β-lactamase and cruzain, respectively.

From Table 1 it can be observed that the β-lactamase model shows in all the compound sets an increased aggregator rate as compared to the cruzain model. This is in contrast to the original training datasets used, which showed comparable aggregator rates for β-lactamase and cruzain assays, although obviously the results obtained here depend on the chemical spaces of the molecules that were aggregators in one or the other training sets. In general, there is a highly significant association between the two models, with a 61% of molecules being aggregators or non-aggregators simultaneously in both models. This rises to 73% in the case of the DrugBank molecules. By considering the intersection between the models, which would correspond to a more robust (although more conservative) prediction of aggregation, the aggregation rate for the whole FooDB would be 39.25% (including 15.34%, 29.76% and 52.98% for the old FooDB, NAG, AG, respectively) vs a 13.32% for the DrugBank.
If we focus on the reliability of the predictions based on the AD of the different sets, we obtain the results collected in Table 2. Again, the number and percentage of molecules within the AD in one or the other model and the intersection of both, for the different compound sets, are shown. We can see very large percentages of molecules within the applicability domain in the updated FooDB (81.96% and 86.57% for β-lactamase and cruzain models, respectively), even higher for the new molecules, especially in the case of AG, which is very close to 100% (98.96% and 99.99%, respectively). For the old FooDB molecules the percentages are lower, 50.93% and 61.46%; these values are similar to those observed for the DrugBank (52.01% and 55.93%).

In this case, the percentage of molecules in the AD is slightly but significantly higher for the cruzain model compared to the β-lactamase model if we consider the whole set of molecules: 85.64% vs 81.05%, respectively. The agreement between both models as far as AD is concerned is very high, and ~94% of the molecules are within or without the AD of both models simultaneously. This is probably due to both models using similar collections of compounds in their training sets, as well as the very high percentages of molecules within the AD in both cases. By intersecting both models, there is just an slight decrease of percentages in all the sets, so that the whole FooDB has a 81.43% (including 49.51%, 78.07%, and 98.96% for old FooDB, NAG, and AG, respectively) vs a 47.58% for the DrugBank.

We could ask what are the SCAM Detective predictions for the compounds marked as known (92) or predicted (37) aggregators by the Aggregator Advisor. Of the 92 known aggregators, 23 are predicted aggregators by the β-lactamase model, and 15 by the cruzain model. Of the 37 predicted aggregators, 16 are predicted aggregators by the β-
lactamase model, while 16 are predicted aggregators by the cruzain model. Thus, there
seems to be a modest agreement for the prediction of the aggregator class between
both SCAM Detective and Aggregator Advisor models, although it must be taken into
account that the datasets were derived in different conditions, with different definitions
of aggregation (different predicted labels), and with different compound sets. In
addition, the numbers used for the comparison are very small, given the tiny number of
compounds predicted by the Aggregator Advisor, and we are only considering the
aggregators class, not the non-aggregators.

In summary, we have obtained novel predictions for the updated FooDB through the
machine learning approach used by the SCAM Detective, which was reported to provide
balanced sensitivity/specificity predictions and an increase of accuracy from 46% to
53%\(^{31}\) compared to other methods (e.g. Aggregator Advisor\(^{12}\) and Hit Dexter\(^{43}\), see
SCAM Detective paper\(^{31}\)). The FooDB show relatively large percentages of predicted
aggregated molecules, 76.70% in β-lactamase and 40.82% in cruzain. The old fraction of
FooDB displays clearly lower percentages (43.17% and 19.85%, respectively), while the
new fraction of molecules shows increased values, especially in the case of AG, which
are predicted aggregators in 95.39% of the cases by the β-lactamase model and 52.98%
by the cruzain one. For the whole FooDB, a very large proportion of molecules appears
to be within the AD of the models, 86.57% for the cruzain model and 81.96% for the β-
lactamase. These predictions, provided as Supporting Information (Table S3), are
expected to help the community of scientist aiming in understanding the biological
mechanisms of action of food compounds to identify aggregators in their assays.

Analysis of interference substructures
In our previous work we also analyzed the presence of nuisance substructures in the FooDB. In this section we repeat that analysis for the updated FooDB. Three filter sets were used, namely PAINS, Glaxo, and LINT. The first one of these was derived by Baell and Holloway\textsuperscript{22} after analysis of a series of high-throughput screens run with the AlphaScreen technology, and comprise a total of 481 filters, grouped in three families with decreasing statistical support: family A (16 filters), corresponding to the filters with the strongest support; family B (55 filters), of filters with median support; and family C, comprising 409 filters with the lowest statistical support. The PAINS filters were derived using a relatively “clean” screening collection developed after an in-silico effort to preclude the presence of inappropriately reactive functional groups, like epoxides, aziridines, alkyl halides, labile esters, etc.\textsuperscript{22} Thus, in order to be able in our analysis for the detection of these substructures, we also included two additional more basic filter sets: Glaxo, corresponding to 55 filters derived in GlaxoWellcome,\textsuperscript{19} and LINT, of 57 filters and generated in Pfizer.\textsuperscript{20}

Figure 4 displays the 18 PAINS filters matched by at least one compound in FooDB, color coded by filter family (green for family A, blue for family B, and red for family C). We can see the same set of filters and almost the same distribution as the one observed previously,\textsuperscript{29} dominated by “catechol_A(92)”, followed by “quinone_A(370)”, “imine_one_A(321)” and “azo_A(324)”. No molecules in the updated FooDB match any more of the 18 matching filters in the old FooDB. The reason is that none of the AG molecules match any of these filters, while just a few set of 74 NAG match a reduced set of three filters observed before:\textsuperscript{29} “catechol_A(92)”, “imine_one_A(321)”, and “quinone_A(370)”. Because of this, the percentage of molecules filtered by PAINS filters
is reduced from 6.80% down to 2.23%. If we focus on the most reliable family A, the percentage of filtered molecules is just a 0.37% (before it was 1.11%), corresponding to ~17% of the total of matches by the PAINS set, while there are 6 filters in this family that match at least one molecule.

Figure 5 shows the matches distribution for the Glaxo filter set. 35 filters match at least one molecule, and now the distribution is overwhelmingly dominated by the first filter, namely “I1 Aliphatic methylene chains 7 or more long”, with a total of 51597 matches. This is because of the new AG molecules, almost all of them matching this filter, as expected due to the frequent presence in their structure of long polymethylenic chains. Other than that, the AG do not match any other Glaxo filter. In the case of the NAG molecules, again the most frequent filter is “I1 Aliphatic methylene chains 7 or more long”, but in this case the second one is no longer “N3 Saponin derivatives”, which is very unusual in these molecules, but instead “I15 Di and Triphosphates”, followed by “I5 Thiols” and “N2 Polyenes”. All in all, no additional Glaxo filter absent in the previous study matches any of the new FooDB molecules.

As regarding the LINT filters (Figure 6), a large increase of matched molecules is observed for the first two filters, “long aliphatic chain, 6+” and “aliphatic ester, not lactones”, as compared to the matches in the previous FooDB, due again to the large number of new AG molecules that match these substructures. No additional filter is matched by the AG compounds, while the NAG ones has as most frequently matched filter “S/PO3 groups”, followed by the previous “long aliphatic chain, 6+”, “alkyl esters of S or P” and “aliphatic ester, not lactones”. As was observed with PAINS and Glaxo, no new filter appears here that were not present in the previous study.
Table 3 collects the filter match statistics for all these filter sets. We can see that, as said before, the new compounds decrease the stringency of both the PAINS and PAINS-A sets, due to the little number of matching molecules in the new FooDB molecules for these sets (74 in total, all of them in the NAG group). The effect of this is to reduce the percentage of matched molecules from 6.80% to 2.23% (PAINS), and from 1.11% to 0.37% (PAINS-A). The contrary is observed for Glaxo and LINT, where the percentage of filtered molecules raises up to 78.45% and 85.11%, respectively, while in the previous work it was 36.18% and 55.43%. Similar dual effect is observed if we focus on the normalized percentage of matched molecules: now it is 0.0046%, 0.023%, 1.44%, and 1.49% for PAINS, PAINS-A, Glaxo, and LINT, while before it was 0.014%, 0.069%, 0.658%, and 0.973%. As in our previous work, the stringency order considering the percentage of filtered molecules is as follows: PAINS-A < PAINS < Glaxo < LINT. If instead we consider this percentage but normalized by the number of filters in the set, PAINS and PAINS-A switch order, but the rest remains the same: PAINS < PAINS-A < Glaxo < LINT. The same order is observed by the fraction of filters with at least one matching molecule in each set, that goes from 18 out of 481 in PAINS, to 49 out of 57 in the case of LINT, with 35 out of 55 in the case of Glaxo in between.

All these filter matches are collected in Supporting Information (Table S4). In the same way, it is expected that this will be useful to identify interferences in biochemical and biological assays of food compounds.
DISCUSSION

A large research effort is being devoted to the determination of the biological mechanism of action of food compounds, in order to understand the beneficial or harmful effect of foods in human health. These studies are conducted by performing biochemical or biological (cellular) assays aiming to see if the food molecule interacts with some biological target, typically a protein. It is well known from the field of drug discovery that in some cases an assay can result in a false positive or misleading outcome due to some property of the tested molecule: the molecule forms colloidal aggregates that denature the target protein or has some substructure that make it prone to membrane disruption, (photo)reactivity, redox cycling, etc., or rather to generate interferences with the assay signal.

In a previous work, we applied cheminformatic techniques from the drug discovery field to identify molecules prone to such false-positive behavior in a database of food compounds, the FooDB, to find food compounds with these putative issues. The FooDB is (quoting from its web site) “the world’s largest and most comprehensive resource on food constituents, chemistry and biology. It provides information on both macronutrients and micronutrients, including many of the constituents that give foods their flavor, color, taste, texture and aroma”. Here we provide an update of that analysis after the FooDB more than duplicated its size (~26K compounds to ~71K compounds), that includes also the use of novel machine learning models to predict aggregation, as the method used before (Aggregator Advisor) was not able to give predictions for the majority of food compounds. We opted to use the so-called SCAM Detective, as it has been observed to yield accuracies ~50% above previous methods, including the
Aggregator Advisor, and as other methods like the Hit Dexter\textsuperscript{43} show lower accuracy\textsuperscript{31} and also predict a slightly different endpoint, namely hit promiscuity, which is a related but different label (aggregators are all promiscuous, but not all promiscuous compounds are such because of aggregating behavior).

From a practical point of view, the files provided here as Supplementary Information should be used by the experimenter to find if the tested compound appears there with one or more annotations for aggregation and/or interference. If the annotation is for aggregation, its presence can be experimentally checked by different approaches: decrease of activity after addition of small quantities of non-ionic detergents, counter-assay in aggregation-sensitive assays (e.g. β-lactamase), or detection of colloidal aggregates through dynamic light scattering. On the other hand, if the annotation is for a substructure that generates assay signal interferences (e.g. absorption, fluorescence, etc.), a possible solution is the test in an orthogonal assay using an alternative technology and signal. The third option are those interfering substructures that provide nonspecific activity (promiscuity) through variable mechanisms (e.g. membrane disruption); in that case it could be possible to run a counter-assay with a different and unrelated target and the same technology. In general, it is always advisable to check in public databases like ChEMBL\textsuperscript{45} or PubChem\textsuperscript{39} about previously reported activities of the compound, which would be informative about possible promiscuity issues if the molecule has shown activity against a wide set of unrelated targets. In addition, if chemical modifications are performed on the compound, finding a lack of a defined structure-activity relationship would be a signature of artifactual activity. More
thorough approaches to these issues have been described elsewhere; and for more specifically referring to publication in ACS journals see reference (28).

The updated prediction performed in this work has increased considerably the number of putative aggregators in FooDB: 77% for the β-lactamase model and 41% for the cruzain model. One reason is the AG component of the new FooDB molecules, for which the β-lactamase model predicts a 95% of aggregators, and the cruzain a 53% (Table 2). Given the very large hydrophobicity of these molecules (median logP of 17.9) together with the extreme flexibility of their aliphatic structure, these predictions seem quite reasonable. The higher hydrophobicity, could also contribute to the increase in aggregator rates in NAG molecules over the old FooDB molecules, together with the significantly larger surface areas of the former group as compared to the latter and the higher number of rotatable bonds. In addition, another reason for getting many more aggregators is that the Aggregator Advisor had not been able to give predictions for the vast majority of the FooDB molecules as just a few of them were similar to one or more in the list of known aggregators: using a Tanimoto radius of 0.85, only 437 in the old FooDB, 20 for NAG, and none for the AG. This shows the advantages of the SCAM Detective, that is trained with a very large and diverse dataset of both aggregators and non-aggregators and gives predictions for the whole chemical space. Also, it is worth mentioning that a very large fraction of the FooDB molecules are within the AD of the SCAM Detective models, for which the predictions are expected to be more reliable, ranging from 51% to 100% depending on the subset and model. Thus, we expect this effort to give a much more reliable and comprehensive identification of putative aggregators in the food molecules of FooDB.
As regarding the interference filter analysis, in the previous work we applied as cheminformatic tool three well-known substructure filter sets derived from the high-throughput screening and medicinal chemistry fields: PAINS, Glaxo and LINT. Here we have applied these filters to the updated FooDB too. As a result, for the PAINS we have observed a very small number of novel FooDB molecules matching them, so that the observed current distribution is very similar to the previous one; also the set of matched filters remains equal. The same set of matched filters are also observed again with the Glaxo and LINT filters, but in this case the distributions change significantly as the filters representing long aliphatic chains or aliphatic esters (“l1 Aliphatic methylene chains 7 or more long” in Glaxo and “long aliphatic chain, 6+” and “aliphatic ester, not lactones” in LINT) have an enormous increase of hits, corresponding mostly to the new AG compounds. As a result, the interference rates for these filter sets increase considerably from the previous analysis, resulting in a total of 78% and 85% for Glaxo and LINT, respectively (before they were 36% and 55%).

In general, we observe a decrease of drug-like properties in the new FooDB molecules, with a significant decrease of its drug-like “chemical beauty” (as measured by the Estimation of Drug-likeness (QED) descriptor, p-value < 0.001 in post-hoc test, see Supplementary Material, Table S2) and an increase of aggregator and interference rates (for the Glaxo and LINT filters). The new FooDB molecules (especially the AG component) tend to have more hydrophobicity, flexibility, and molecular weight; these factors make them more prone to aggregation on one hand (also the higher TPSA in the NAG molecules), and to display the long polymethylene-type of interfering substructure appearing in the Glaxo and LINT filter sets. The near complete absence of interferences
of the PAINS filters can on the other hand be explained by considering that the later
were derived from a collection of relatively “clean” compounds previously filtered from
more basic problematic substructures, to make them more amenable as starting points
for drug development. The PAINS filters derived from that collection would be more
specific to drug- or lead-like molecules, probably of a more synthetic origin, and
corresponding to substructures scarcely present in the FooDB.

To conclude, we can say that the putative aggregators and interference matches for the
new FooDB found in this work and available as Supporting Information would help to
decrease the false positives in assays performed with food compounds, by applying,
when present, the approaches discussed above, and therefore to gain a better and more
robust understanding of their biological mechanisms of action, thus reducing the rates
of false positive results in public databases like ChEMBL\textsuperscript{45} or PubChem\textsuperscript{39}. Other uses for
these predictions would be the prioritization of compounds for testing, applications in
large-scale data mining efforts for understanding structure-activity relationships, design
of reliable nutraceuticals, and selection of novel scaffolds for development of new drugs.
ABBREVIATIONS

American Chemical Society (ACS), Small, Colloidal, Interfering Molecules (SCAM)
Detective, Pan-Assay INterference compoundS (PAINS), Invalid Metabolic PanaceaS (IMPs)

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Notes
The authors declare no competing financial interest.

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SUPPORTING INFORMATION

Statistical Analysis of PhysChem Distributions.xlsx. Both p.values for tests for comparison of physicochemical distributions between groups of compounds (omnibus and pairwise post-hoc), and descriptive statistics (median + interquartile range) for these distributions are collected here.

SCAM Detective Predictions for FooDB.xlsx. Aggregator predictions for the FooDB using the SCAM Detective (both β-lactamase and cruzain models). The prediction result (0 non-aggregator, 1 aggregator) plus the AD result (Inside AD vs Outside AD) is shown for each FooDB compound.

Filter Matches for FooDB.xlsx. Filter matches for PAINS, Glaxo and LINT filter sets for the updated FooDB
REFERENCES


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1. Figure 1. Boxplots for distributions of TPSA (topological polar surface area), LOGP (logarithm of the octanol/water partition coefficient), RB (number of rotatable bonds), HBD (number of hydrogen bond donors), HBA (number of hydrogen bond acceptors), MW (molecular weight), QED (quantitative estimation of drug-likeness), NRING (number of rings), and FSP3 (fraction of sp3-hybridized carbons), for the previous release of FooDB analyzed before\textsuperscript{29} (FDB(OLD)), new non-acylglycerol FooDB molecules in the new release (FDB(NEW/NAG)), new FooDB acylglycerol molecules (FDB(NEW/AG)), and DrugBank molecules (DB). For clarity purposes, outliers have been removed from the plots.

2. Figure 2. Bemis-Murcko\textsuperscript{36,37} scaffold distributions (10 top scaffolds only shown in decreasing frequency) for old FooDB, new FooDB (NAG), and DrugBank molecules.

3. Figure 3. Fragment contribution maps for SCAM Detective predictions for FDB00135 (from the former FooDB) and FDB080642 (a novel AG) in the β-lactamase and cruzain models. The former compound is a predicted non-aggregator in both models, while the later is a predicted aggregator in both models. The color and concentration of contours indicate the direction and strength of the contribution: magenta for aggregation, and green for non-aggregation. Strong contributions result in concentrated contours, weak in separated ones.

4. Figure 4. PAINS filter set distribution across FooDB matching molecules. Only the 18 filters with at least one match are displayed. Bars are color coded by filter family, where family A is green, family B is blue, and family C is red.
5. Figure 5. Glaxo filter set distribution across the FooDB matching molecules. Only the 35 filters with at least one match are displayed.

6. Figure 6. LINT filter set distribution across the FooDB matching molecules. Only the 49 filters with at least one match are displayed.
Table 1. Statistics of Prediction of Aggregators by the SCAM Detective

<table>
<thead>
<tr>
<th>Compound Set</th>
<th>β-lactamase</th>
<th>cruzain</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>FooDB</td>
<td>54147 (76.70)</td>
<td>28815 (40.82)</td>
<td>27707 (39.25)</td>
</tr>
<tr>
<td>FooDB(OLD)</td>
<td>10124 (43.17)</td>
<td>4656 (19.85)</td>
<td>3598 (15.34)</td>
</tr>
<tr>
<td>FooDB(NEW/NAG)</td>
<td>2608 (69.94)</td>
<td>1160 (31.11)</td>
<td>1110 (29.76)</td>
</tr>
<tr>
<td>FooDB(NEW/AG)</td>
<td>41415 (95.39)</td>
<td>22999 (52.98)</td>
<td>22999 (52.98)</td>
</tr>
<tr>
<td>DrugBank</td>
<td>755 (34.44)</td>
<td>414 (18.89)</td>
<td>292 (13.32)</td>
</tr>
</tbody>
</table>

For the different compound sets, the number (percentage) of predicted aggregators in the β-lactamase and cruzain models, and the intersection of both models, are shown.
Table 2. Molecules Within Applicability Domain of SCAM Detective Models\(^a\)

<table>
<thead>
<tr>
<th>Compound Set</th>
<th>β-lactamase</th>
<th>cruzain</th>
<th>Both</th>
</tr>
</thead>
<tbody>
<tr>
<td>FooDB</td>
<td>57859 (81.96)</td>
<td>61113 (86.57)</td>
<td>57487 (81.43)</td>
</tr>
<tr>
<td>FooDB(OLD)</td>
<td>11945 (50.93)</td>
<td>14415 (61.46)</td>
<td>11611 (49.51)</td>
</tr>
<tr>
<td>FooDB(NEW/NAG)</td>
<td>2950 (79.11)</td>
<td>3288 (88.17)</td>
<td>2912 (78.07)</td>
</tr>
<tr>
<td>FooDB(NEW/AG)</td>
<td>42964 (98.96)</td>
<td>43410 (99.99)</td>
<td>42964 (98.96)</td>
</tr>
<tr>
<td>DrugBank</td>
<td>1140 (52.01)</td>
<td>1226 (55.93)</td>
<td>1043 (47.58)</td>
</tr>
</tbody>
</table>

\(^a\) For the different compound sets, the number (percentage) of compounds within the applicability domain in the β-lactamase and cruzain models, and the intersection of both models, are shown.
<table>
<thead>
<tr>
<th>Filter Set</th>
<th># filters</th>
<th># matching filters</th>
<th># matching molecules</th>
<th>Filtered molecules (%)</th>
<th>Filtered molecules / filter (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAINS</td>
<td>481</td>
<td>18</td>
<td>1554</td>
<td>2.23</td>
<td>0.0046</td>
</tr>
<tr>
<td>PAINS-A</td>
<td>16</td>
<td>6</td>
<td>260</td>
<td>0.37</td>
<td>0.023</td>
</tr>
<tr>
<td>Glaxo</td>
<td>55</td>
<td>35</td>
<td>54693</td>
<td>78.45</td>
<td>1.44</td>
</tr>
<tr>
<td>LINT</td>
<td>57</td>
<td>49</td>
<td>59337</td>
<td>85.11</td>
<td>1.49</td>
</tr>
</tbody>
</table>

*aFor the sets PAINS, PAINS-A, Glaxo, and LINT, the number of filters, number of matching filters, number of matching molecules, filtered molecules (%), and filtered molecules per filter (%) are displayed for the updated FooDB compound set.*
Figure 1.
Figure 2.
Figure 3.
Figure 4.
Figure 5.

Filter set distribution in FOODB

- I1 Aliphatic methylene chains 7 or more long
- N3 Saponin derivatives
- I15 Di and Triphosphates
- I7 2,4,5 trihydroxyphenyl
- I6 Epoxides, Thioepoxides, Aziridines
- N2 Polynes
- I5 Thiols
- I4 Disulphides
- I2 Compounds with 4 or more acidic groups
- N1 Quinones
- R18 Quaternary C, Cl, I, P or S
- R7 Peroxides
- R11 Isocyanates & Isothiocyanates
- N6 Monensin derivatives
- R1 Reactive alkyl halides
- R16 beta carbonyl quaternary Nitrogen
- I8 2,3,4 trihydroxyphenyl
- R21 Nitroso
- R6 Acid anhydrides
- I11 Benzyl quaternary Nitrogen
- I12 Thioesters
- N8 Squalestatin derivatives
- I16 Beta lactams
- R17 acylhyrazide
- I10 Thiocyanate
- R26 Cyanohydrins
- R4 Sulphate esters
- I14 Four membered lactones
- R22 P/S Halides
- R2 Acid halides
- R31 Azoalkanals
- I3 Crown ethers
- R5 Sulphonates
- R12 Triflates
- N4 Cytochalasin derivatives
Figure 6. TABLE OF CONTENTS GRAPHIC

FooDB (Database of Food Small Molecules) → Cheminformatic Tools

- Aggregator
- Interference substructure

Assay false positive? Promiscuity?

Orthogonal Assay Counter Assay Literature Review Etc.