

Introduction

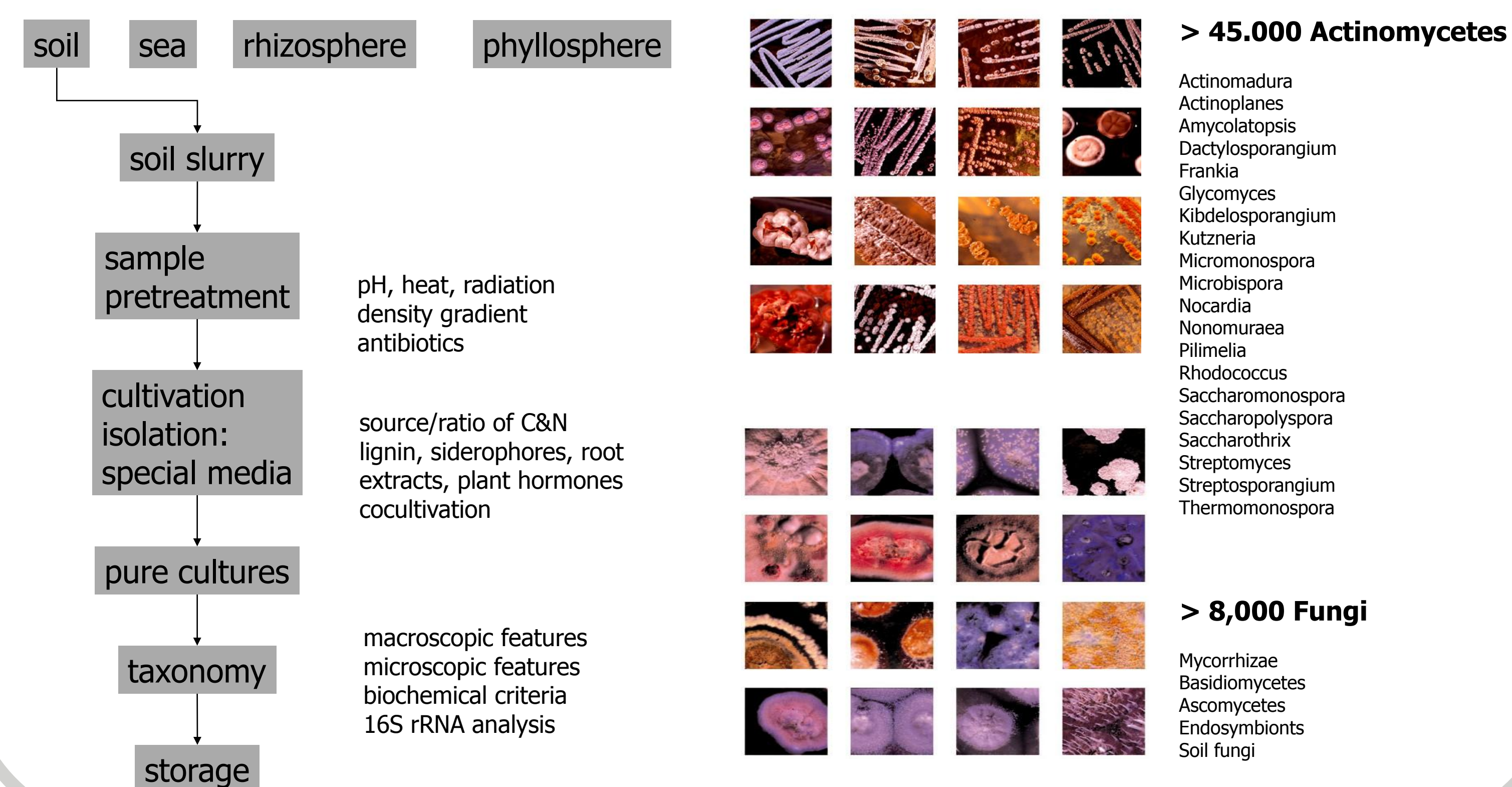
Despite past successes of natural product drugs, most recent natural product hit finding programs have not delivered as expected.

We have performed more than 25 screening campaigns using our library of pre-purified natural product extracts - subfractions - and the associated technical platform for the identification of active natural compounds. The screens covered a broad range of target classes and assay formats and identified over 600 compounds.

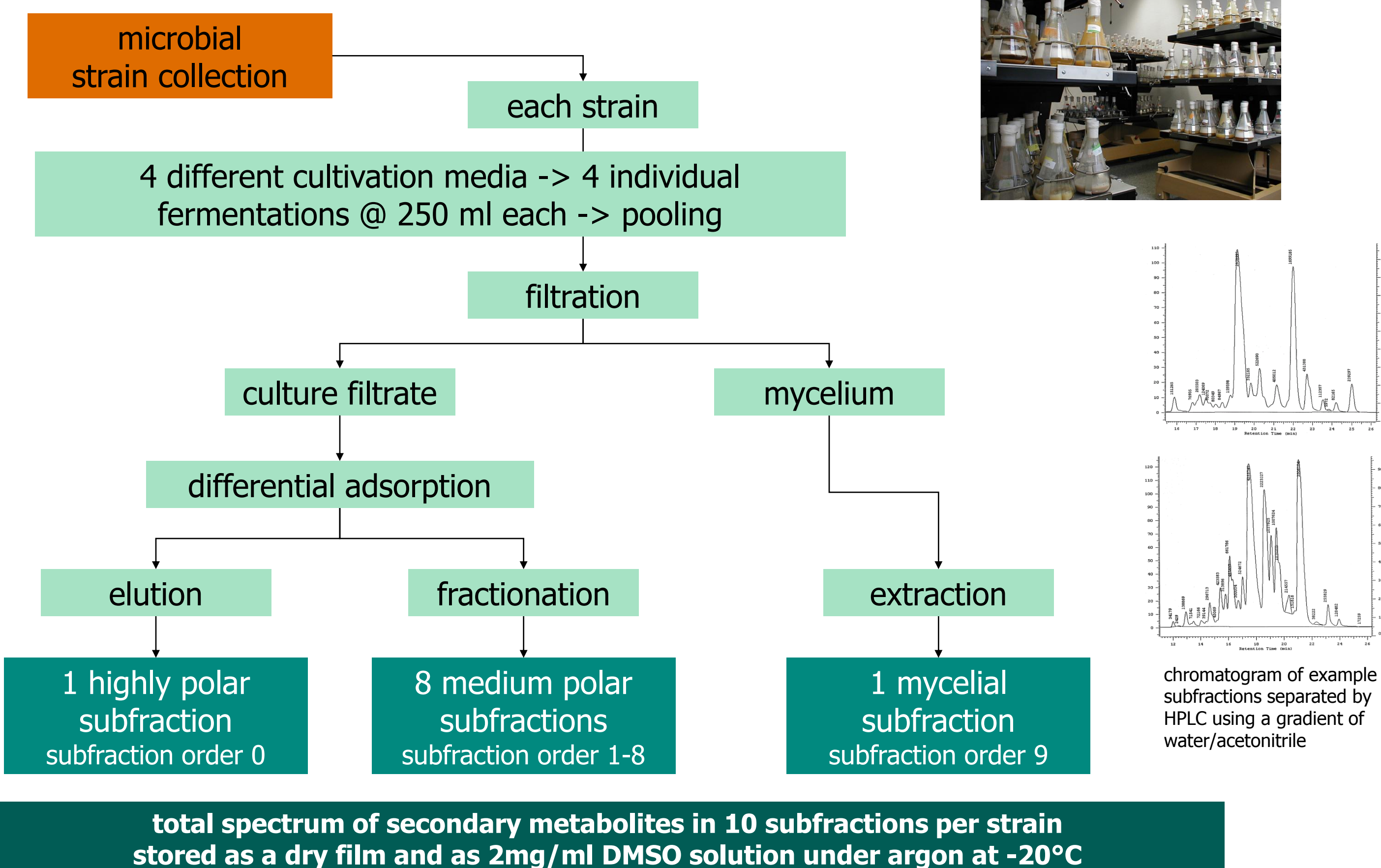
The present poster will reveal how we created the library and discuss how individual parameters affect the outcome of the hit finding campaign.

We will show a detailed analysis of the screening campaigns - on the level of active subfractions as well as identified compounds - in terms of biochemical diversity, selectivity, novelty, structural diversity and physicochemical properties.

Microbial strain collection



Subfraction generation



Subfraction HTS hits: Analysis of diversity, selectivity and strain origin

A total of 27 screens have been performed with subfractions (SFs). All 27 screens have been analyzed with regard to polarity of the hit SFs (figure 1 and 2) and the fungal or bacterial origin of the hit SFs (table 2). A subset of 15 screens with a sufficiently large overlap was analyzed with regard to selectivity of the SFs (table 1) and the origin of the source microorganisms (figures 3 and 4). Details of the screens including hit rates are summarized in table 3.

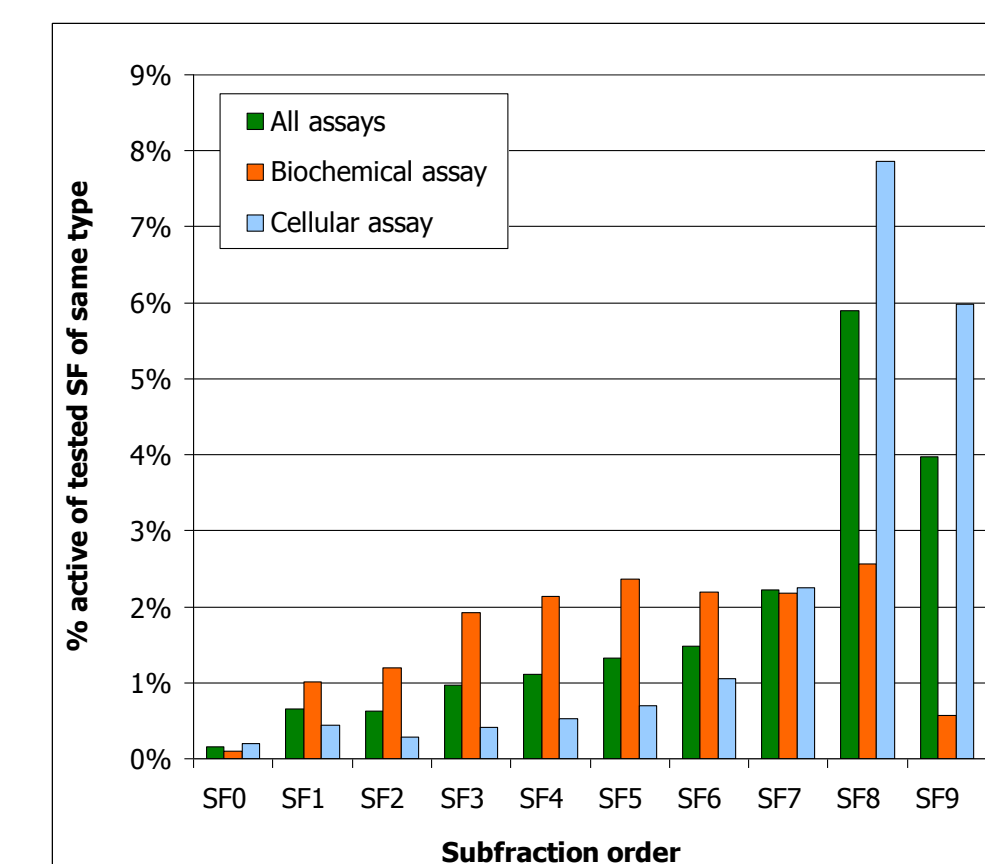


Figure 1: Hit rates and subfraction order (polarity). Comparison of assay types.

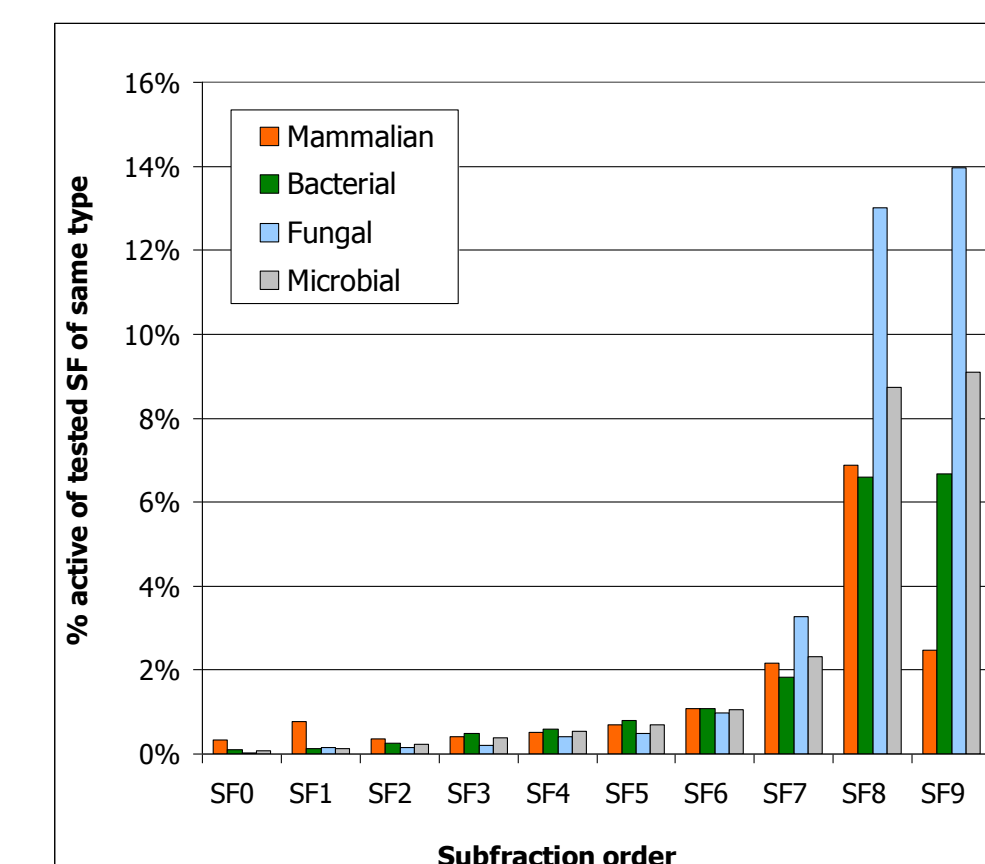


Figure 2: Hit rates and subfraction order (polarity). Comparison of cellular assay types.

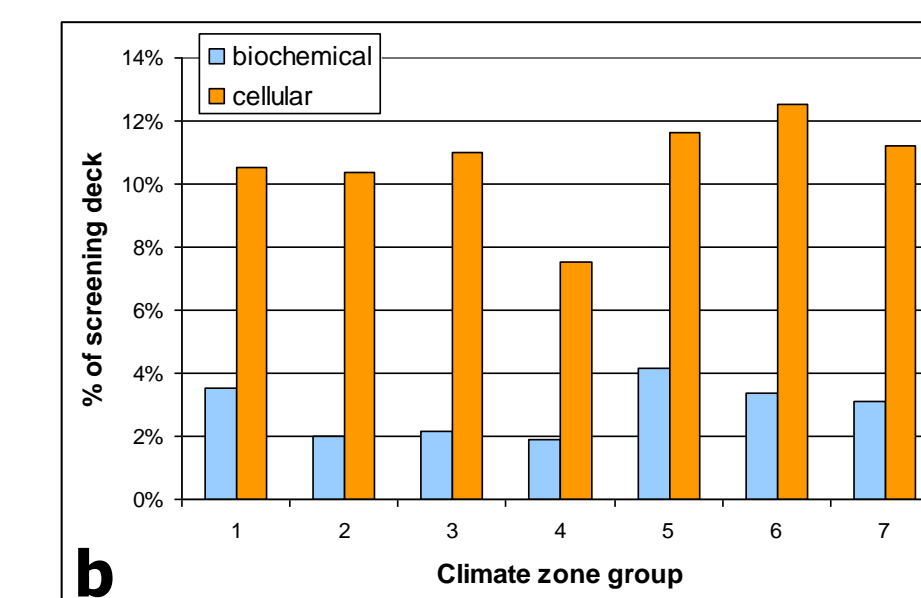
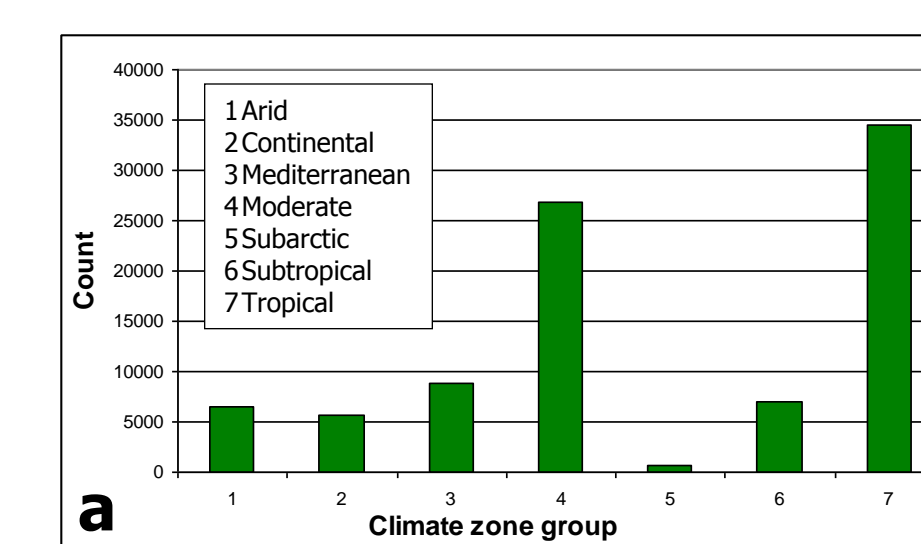


Figure 3a: Origin of the source microorganisms. b. Distribution of SF hits over the source climate zones.

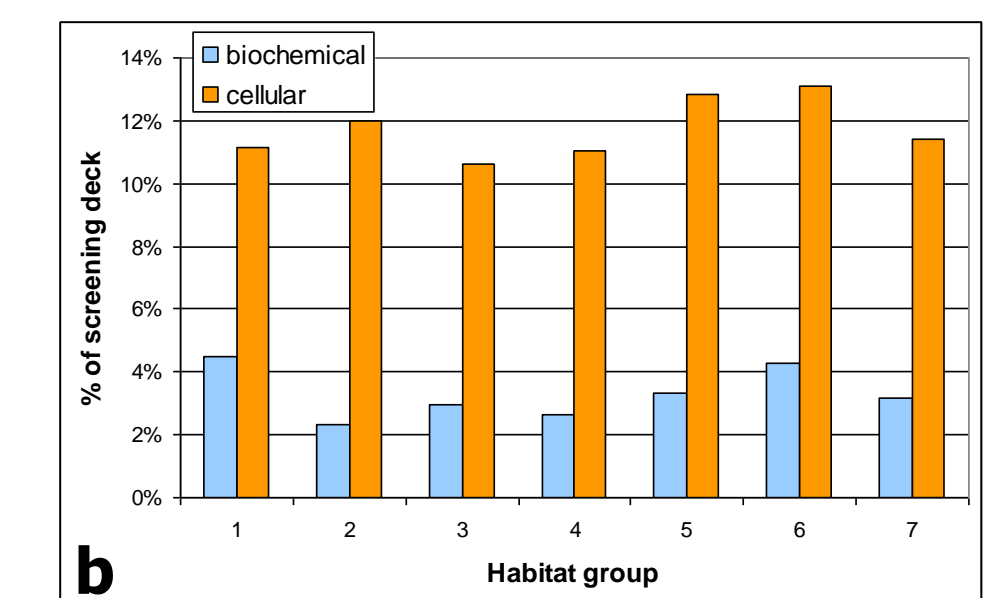
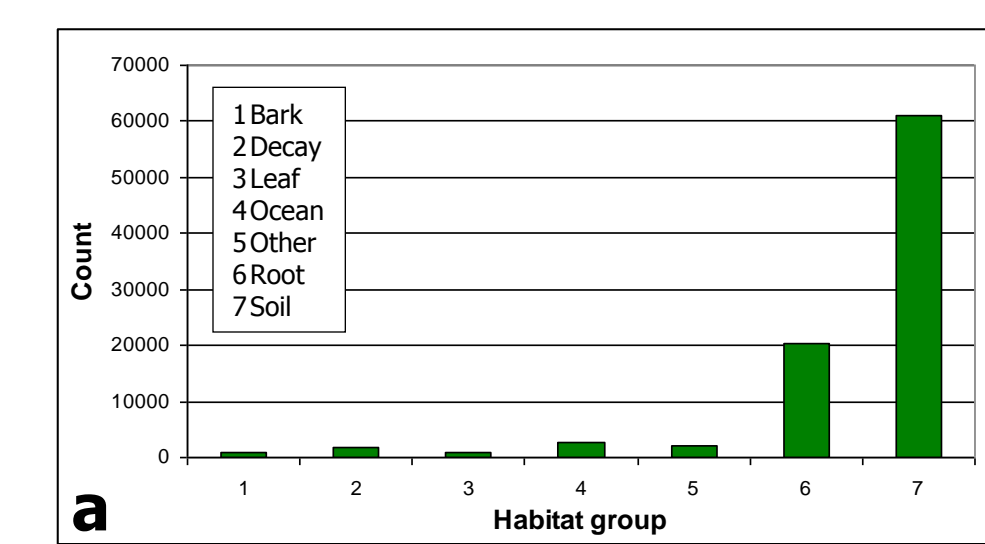


Figure 4a: Origin of the source microorganisms. b. Distribution of SF hits over the source habitat groups.

screen ID	targetclass	assaytype	cell assay subgroup	% hits	analyzed for fig. 3a2 & 3b1a	analyzed for fig. 3a4 & 3b1b
314	antibacterial	cellular	bact	0.2	x	x
311	antibacterial	cellular	bact	5.1	x	x
309	antibacterial	cellular	bact	0.2	x	x
302	antibacterial	cellular	bact	1.6	x	x
303	antibacterial	cellular	bact	2.3	x	x
416	antibacterial	cellular	bact	1.1	x	x
341	antifungal	cellular	fungal	4.3	x	x
341	antifungal	cellular	fungal	2.3	x	x
341	antifungal	cellular	fungal	3.1	x	x
0059	GPCR	cellular	mam	1.5	x	x
0050	GPCR	cellular	mam	1.9	x	x
0050	GPCR	cellular	mam	2.7	x	x
368	channel	cellular	mam	1.5	x	x
396	pathway	cellular	mam	0.8	x	x
373	antimicrobial	cellular	mam	0.8	x	x
364	pathway	cellular	mam	2.5	x	x
392	antiviral	cellular	mam	0.3	x	x
0010	kinase	biochem	n.s.	2.3	x	x
0010	kinase	biochem	n.s.	2.4	x	x
0010	phosphatase	biochem	n.s.	1.6	x	x
001	protein protease	biochem	n.s.	0.3	x	x
0020	protease	biochem	n.s.	4.0	x	x
0030	protease	biochem	n.s.	3.3	x	x
321	protease	biochem	n.s.	0.1	x	x
321	protease	biochem	n.s.	0.5	x	x
366	pathway	biochem	n.s.	1.9	x	x
391	pathway	biochem	n.s.	0.9	x	x

Table 3: Individual hit rates and details of the screens analyzed

hit frequency	all screens	bio-chemical	cellular	mammalian	bacterial
1	8738	2682	7744	4397	5483
2	1629	128	1421	599	895
3	717	7	613	77	404
4	379		297	2	152
5	180		158		41
6	118		99		7
7	80		23		
8	31		2		
9	6				
total	11878	2817	10357	5075	6982
screens	15	4	11	5	6

Table 1: Hit selectivity. The table shows the number of subfractions in the different assay types that were active with a certain frequency (hit frequency), i.e. in 1, 2, 3, ..., 9 assays. These numbers provide an indication of hit selectivity.

assay types	average hit rate from fungal strains (%)	average hit rate from bacterial strains (%)
all	6.5	9.9
biochemical	5.9	4.9
cellular	6.8	12.9
anti-bacterial	7.6	13.3
anti-fungal	5.6	20.2
mammalian cells	6.6	9.9

Table 2: Hit rates in SFs from fungal and bacterial origin. The table shows the percentage of fungal and bacterial strains that produced an active subfraction in the different assay classes.

Conclusions

- In biochemical screens SFs of a broad polarity range are active, whereas in cellular assays hits concentrate in the non-polar fractions, as expected.
- Hits are equally distributed over the different habitats and climate zones of the source microorganisms.
- In biochemical assays the subfraction selectivity appears to be high; in more complex (cellular) assays SFs exhibit lower selectivity; frequent SF hitters are scarce.
- Bacterial source strains delivered more hits in cellular assays (especially antimicrobial) compared to fungal source strains; in biochemical screens fungal and bacterial source strains delivered approximately similar hit rates.
- The SF screens delivered hits in all projects performed so far.

Recommendations

- BioFocus' subfraction collection is well suited to deliver hits with good selectivity in a broad range of assay formats and target classes.
- BioFocus' natural product libraries complement synthetic screening libraries. Our subfraction collection should therefore be considered for hit finding programs, where chemical diversity and/or novelty is important.
- BioFocus' subfraction collection has not been exploited in certain target classes and therapeutic areas, such as protein-protein interactions and oncology. We expect that a high degree of novelty and IP space will be generated in such hit finding projects.
- An additional observation/recommendation from our work (not described in the results above) is that with complex screening assays (cellular, whole cell antimicrobial) a good screening cascade is required to eliminate subfractions with well known/cytotoxic/other unwanted components early in the process.

Identified natural products: Analysis of diversity, physico-chemical properties and novelty

645 natural compounds have been identified during the follow up of subfraction screening programs. Their chemical properties have been analyzed as shown below.

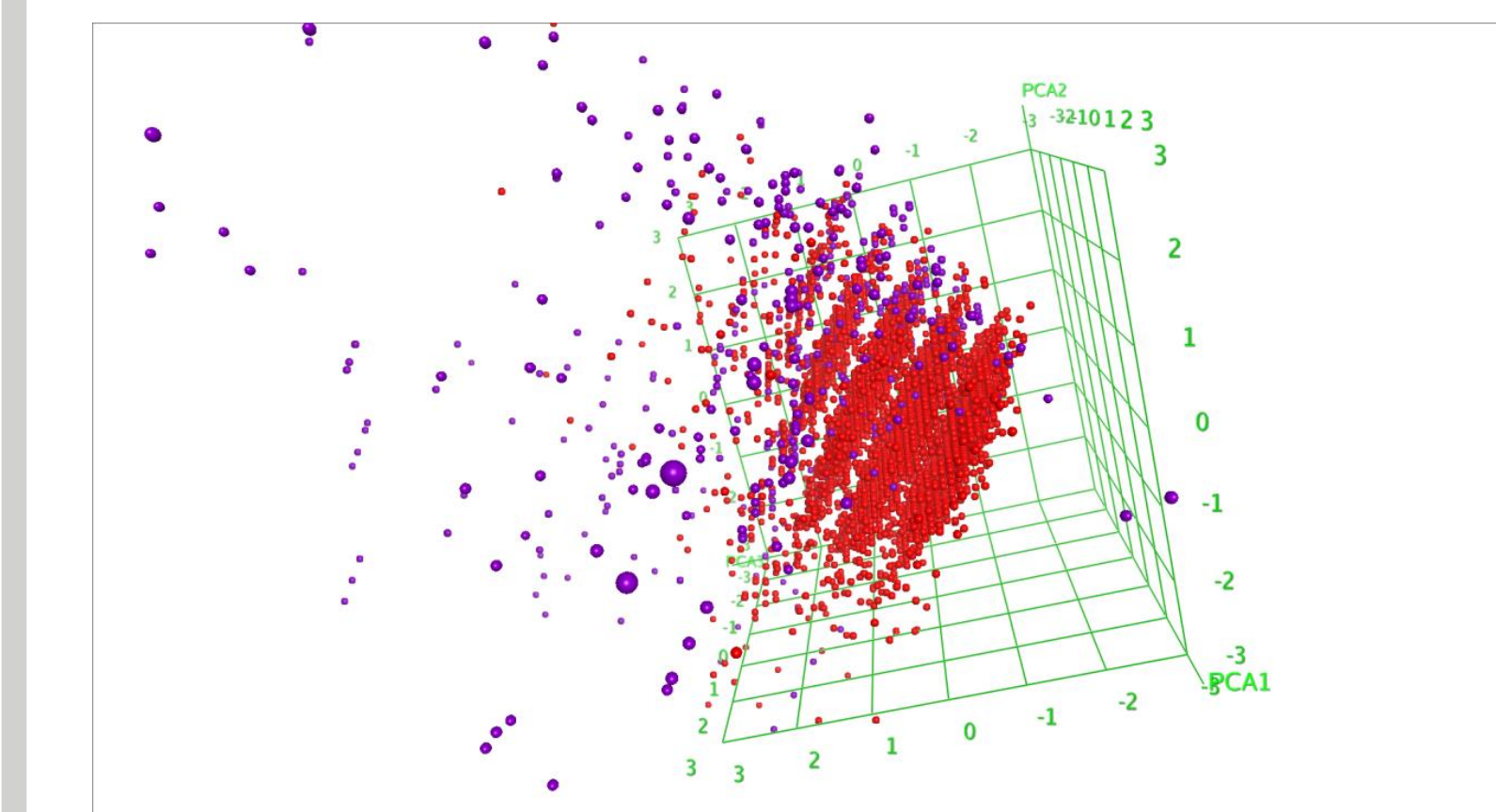


Figure 5: Diversity comparison of the collection of 645 natural compounds (purple) with BioFocus' collection of synthetic compounds (red). A principal component analysis was performed using the number of H-bond donors, H-bond acceptors, the number of rotatable bonds, and the atom-count. Three principal components are plotted in the diagram.

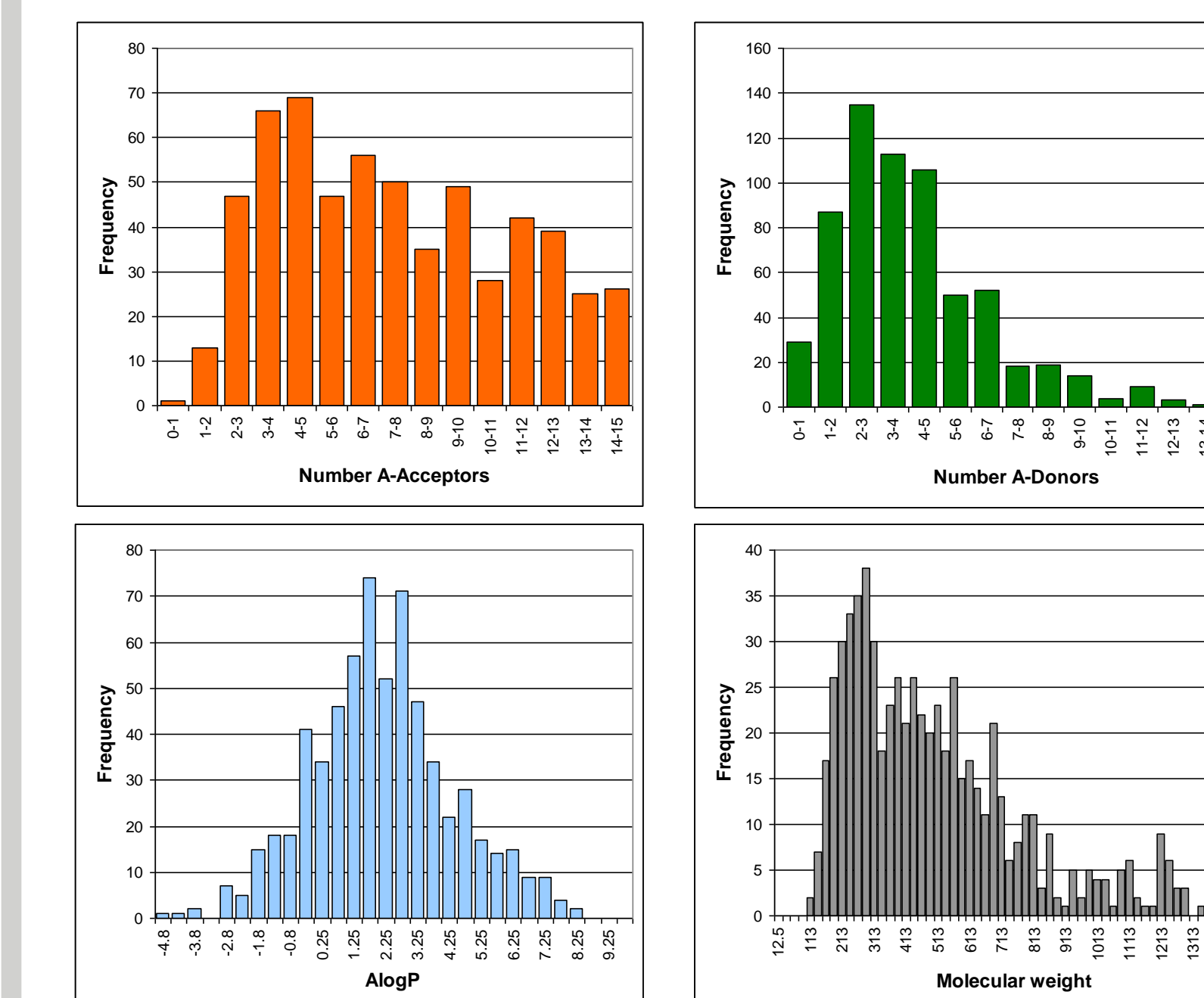


Figure 6: Distribution plots of the 4 "Lipinski properties" determined for the 645 identified natural compounds.

Novelty

Searches in natural product databases revealed that 135 of the 645 identified natural compounds have not been described before, i.e. are novel compounds (21%).

Conclusions

- The diversity analysis showed, that BioFocus' natural compound collections complement the BioFocus diverse library of synthetic compounds.
- The natural compounds cover a broad range of physicochemical properties, even beyond the "Lipinski room". Lipinski's rule of five is not directly applicable to natural compounds and many natural compounds, that do not obey Lipinski's rule of five, are orally bioavailable (Curr Opin Chem Biol 2008, 12, 306).
- More than 20% of the natural compounds discovered in the screens are novel, i.e. deliver valuable IP.