Applying BioAssay Ontology to facilitate HTS analysis

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Assay Informatics project

Innovative Medicine Initiative is an EC funded public-private partnership for pharmaceutical research

Create an *"Open Pharmacological Space"* to lower costs and drive benefits from interoperable drug-discovery data

- Common language for assay annotation

- Improved project success analyses based on assay technologies

- Better understand the impact of technology artefacts like frequent hitters

- Assay design and screening cascade support during assay development in early projects

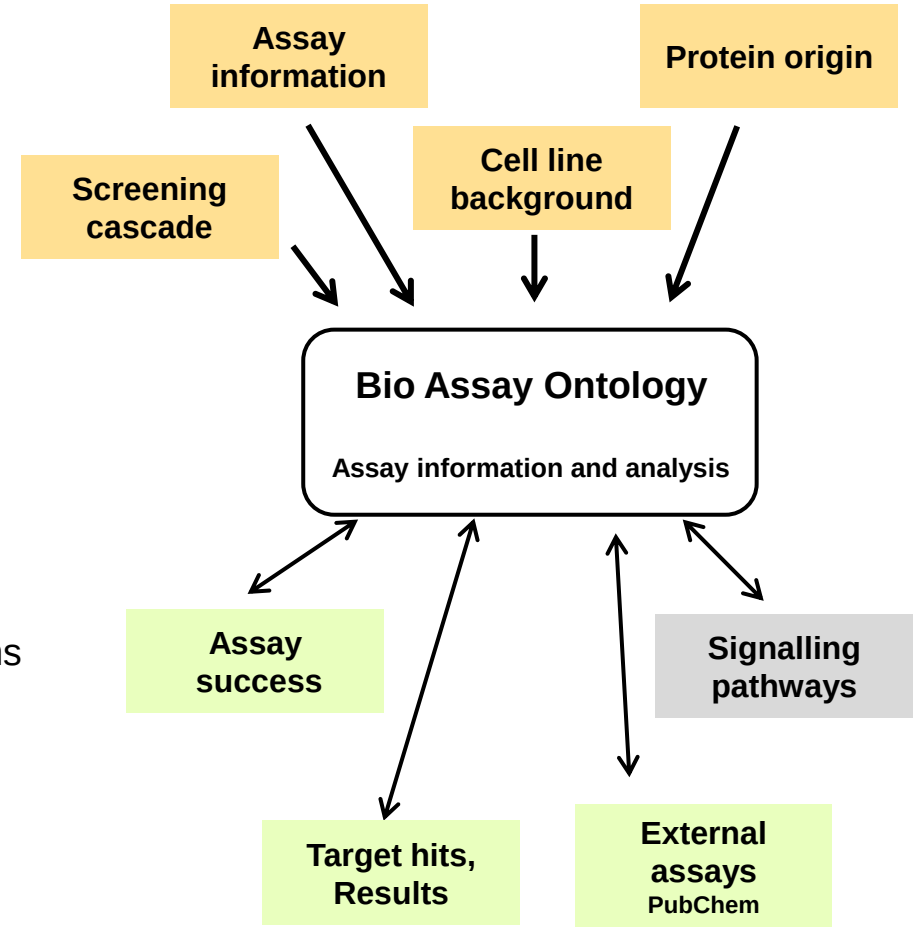
- Improved capability to perform combined data mining of internal and public data

- **BioAssay Ontology**
- **Assay development support**
- **Frequent hitter analysis**

BioAssay Ontology

Computational Science, University of Miami, USA

- **Assay design**
- **Assay format**
- **Detection technology**
- **Meta target**
- **Endpoint**
- **Perturbagen**



BioAssay Ontology has imported sections from:

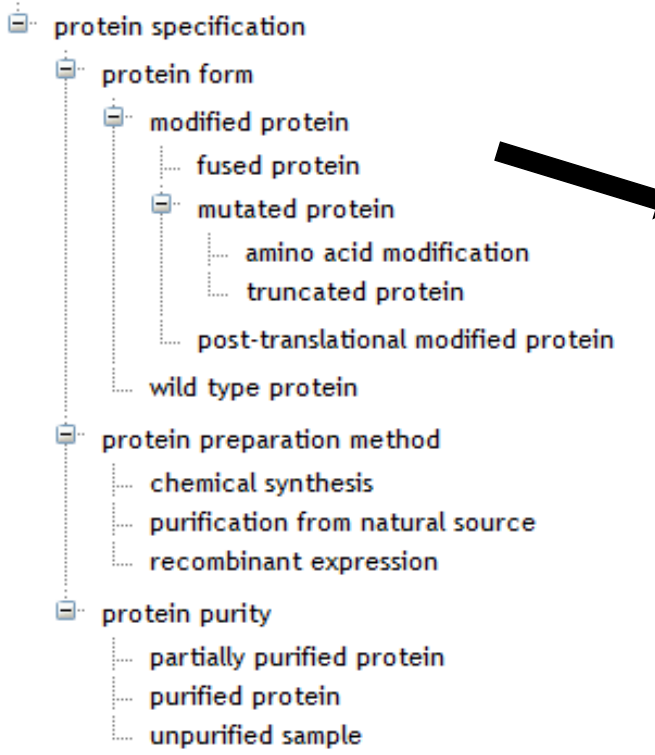
NCBI taxonomy - organism names and IDs
Uniprot - protein target names and IDs
Unit Ontology - concentration and time unit terms
Ontology of Biomedical Investigation – descriptions of biological assays
Gene Ontology - biological processes
Cell Line Ontology - cell line names
CL – cell types
UBERON – anatomical entities
PATO – cell phenotype
SAR connect – target classifications

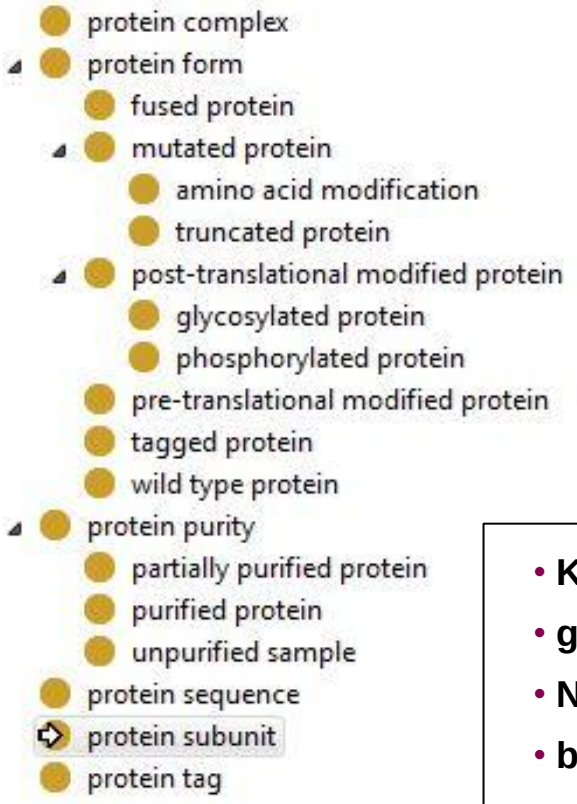
(Eriksson M. et.al. Mol Inform. 2012 Aug;31(8):555-568)

BioAssay Ontology

Evaluation and modification of the ontology

Does the protein origin, such as the post-translational modification effect the technology?





- **KEGG family ID**
- **gram + / - / atypical**
- **NCBI Taxonomy classes**
- **bacterial strain**

BioAssay Ontology

Annotation of HTS assays

HTS assay: reporter gene assay

- Assay method: reporter gene method: beta lactamase induction
- Detection technology: FRET
- Bioassay: beta lactamase assay

- *Assay kit: LiveBLAzer FRET - B/G Loading Kit*
- *Wavelength: ex 405 em 460, 535*

- Biological process
- Disease

HTS assay: FLIPR

- Assay method: molecular redistribution determination assay
- Detection technology: fluorescence intensity
- Bioassay: calcium redistribution assay

- *Assay kit: Fluo-8 No Wash Calcium Assay Kit*
- *Wavelength: ex 480 em 530*

- Biological process
- Disease

Manual annotation of protocols

Over 900 PubChem assays have been annotated by the BioAssay Ontology team

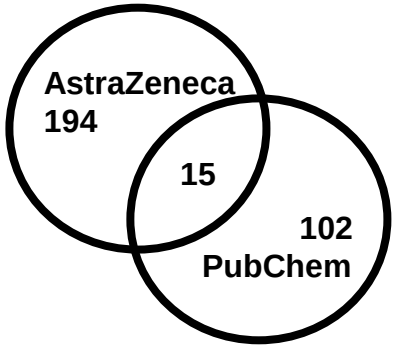
Assay development support

Comparison study between AstraZeneca and PubChem HTS assays

412 in-house HTS assays since 2005 have been annotated according to the BioAssay Ontology. The assay design and technology of the annotated assays were analyzed together with 239 primary assays from PubChem. The analyzed PubChem assays are biochemical assays, assays detected by luminescence and/or assays using GPCR targets.

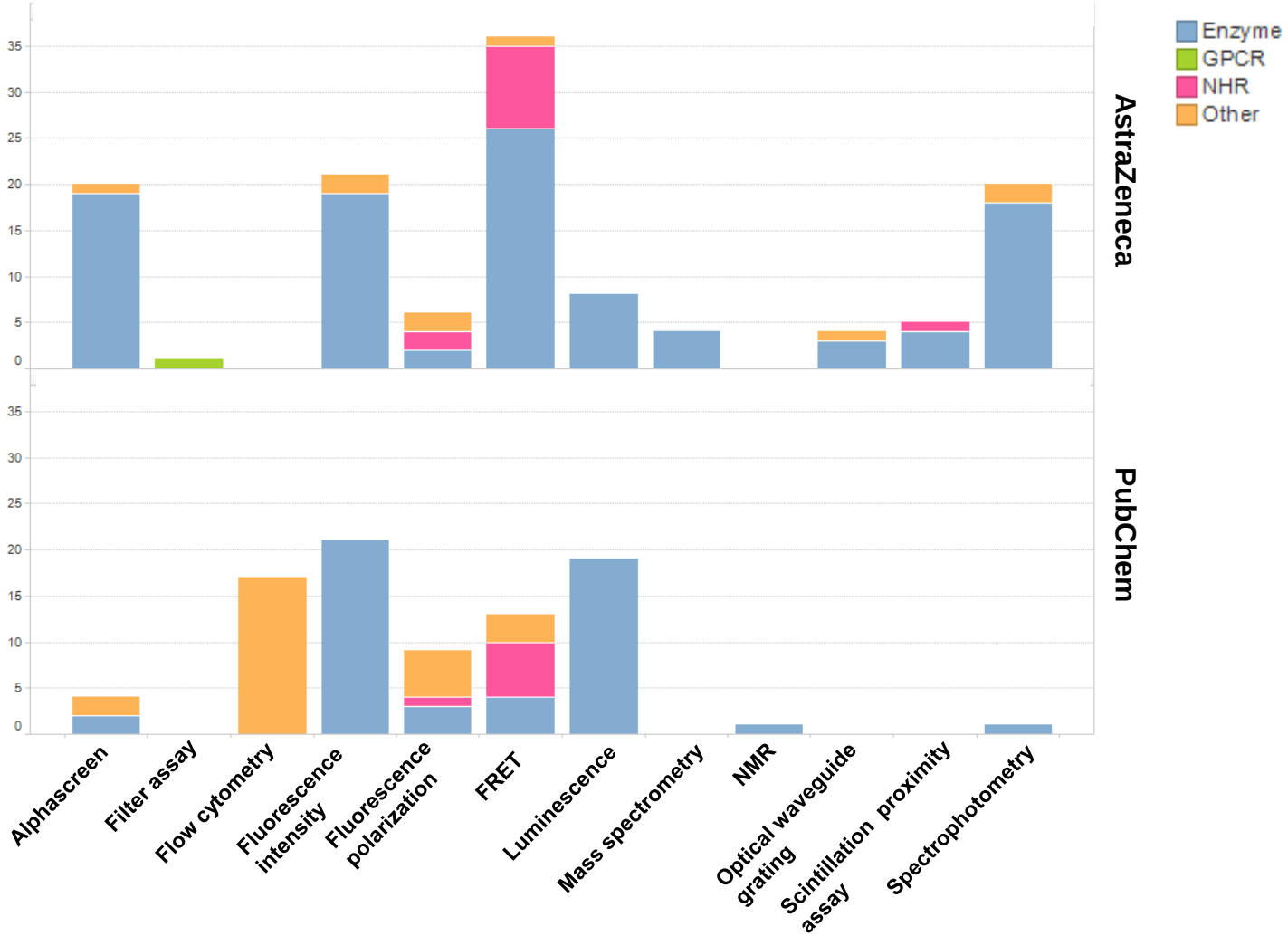
From the annotated assays, 515 assays were using human targets and combined 311 different human targets were represented in the study.

15 of the in-house targets were also screened in at least one PubChem assay. Eight of these were GPCR targets.



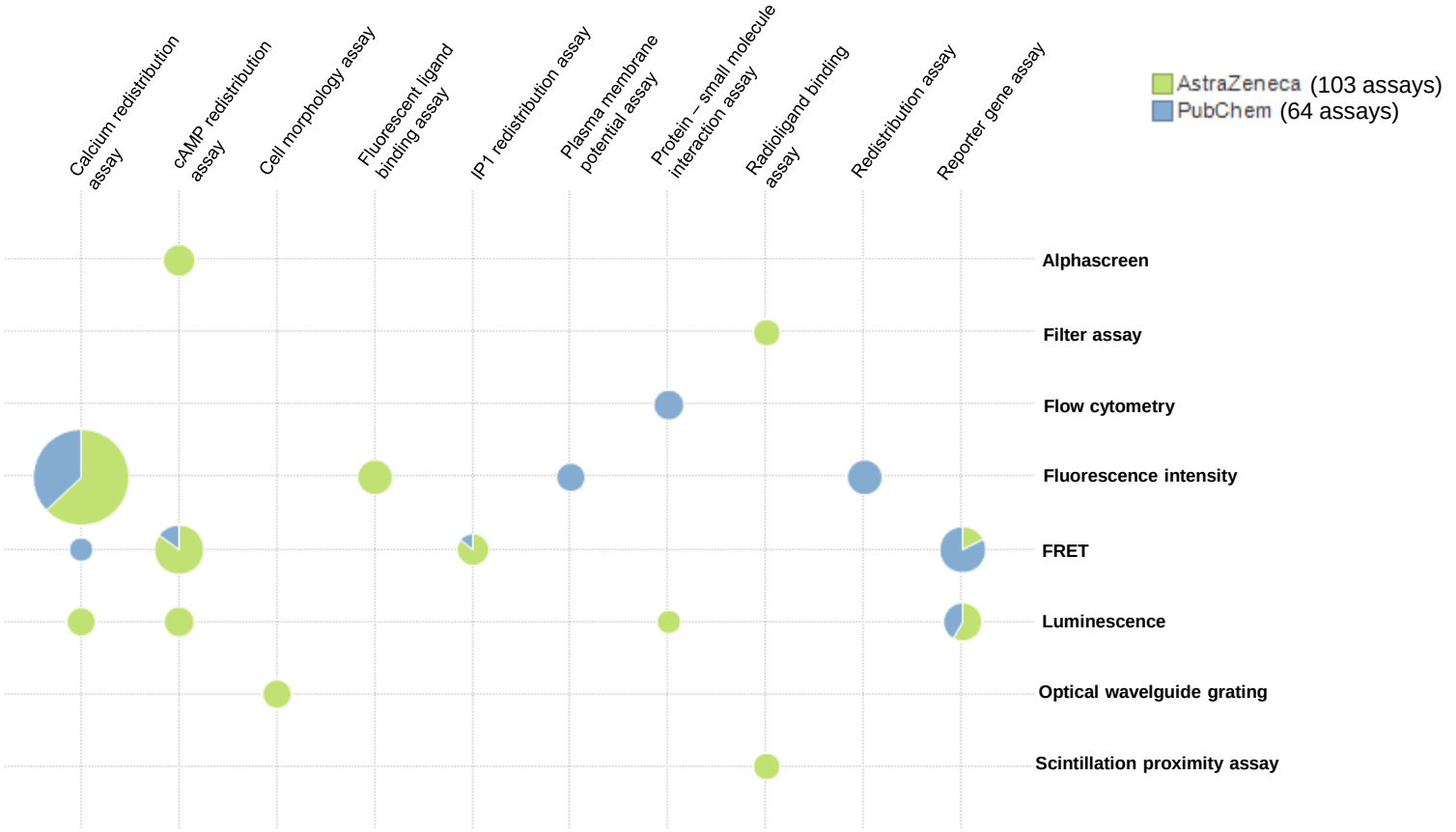
Assay development support

Detection technology of AZ and PubChem biochemical assays



Assay development support

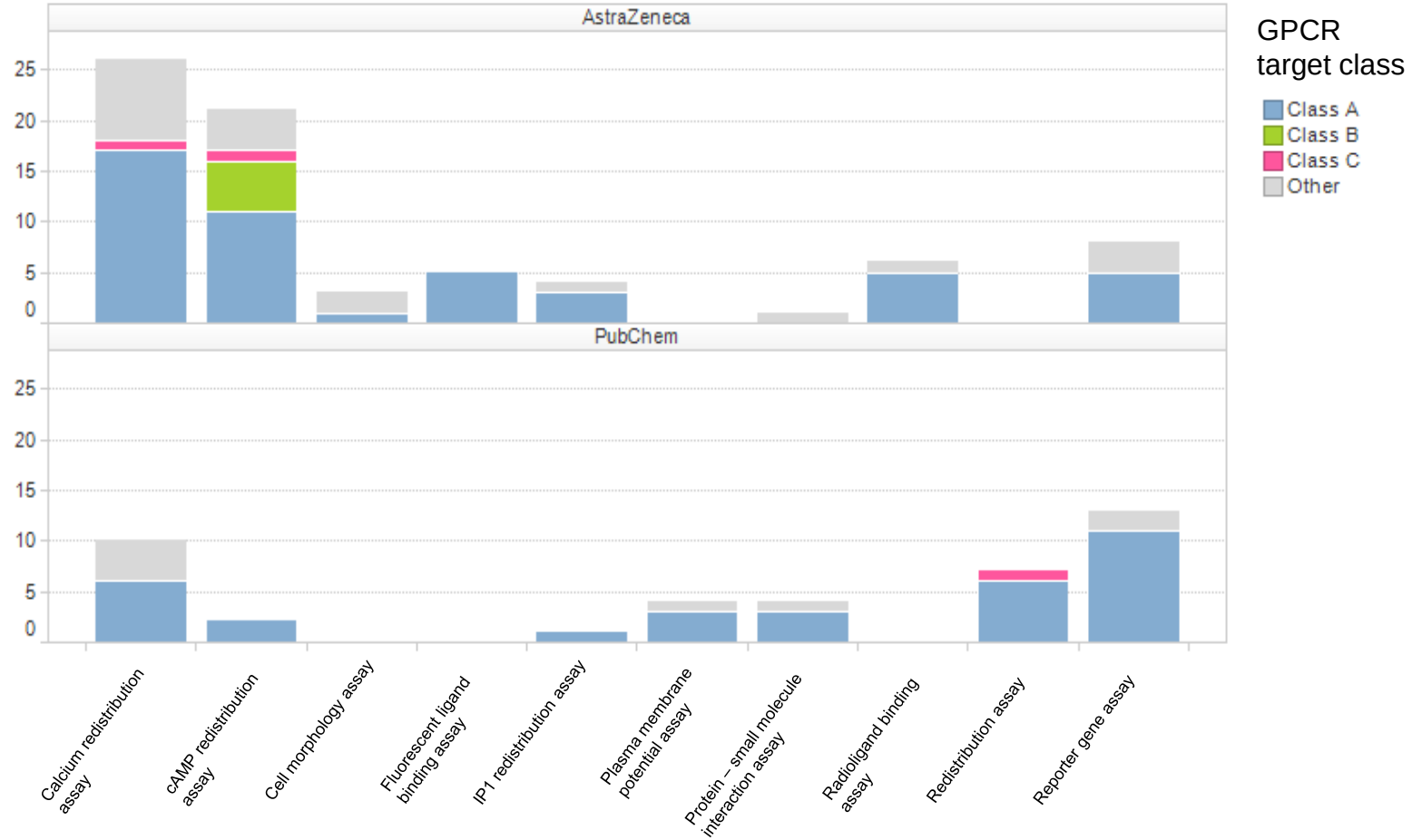
Assay design and technology of AZ and PubChem GPCR assays



cAMP redistribution and fluorescent and radioligand assays seems to be more regularly used within AstraZeneca than what is published in PubChem. In assays published in PubChem the reporter gene method is more frequently used.

Assay development support

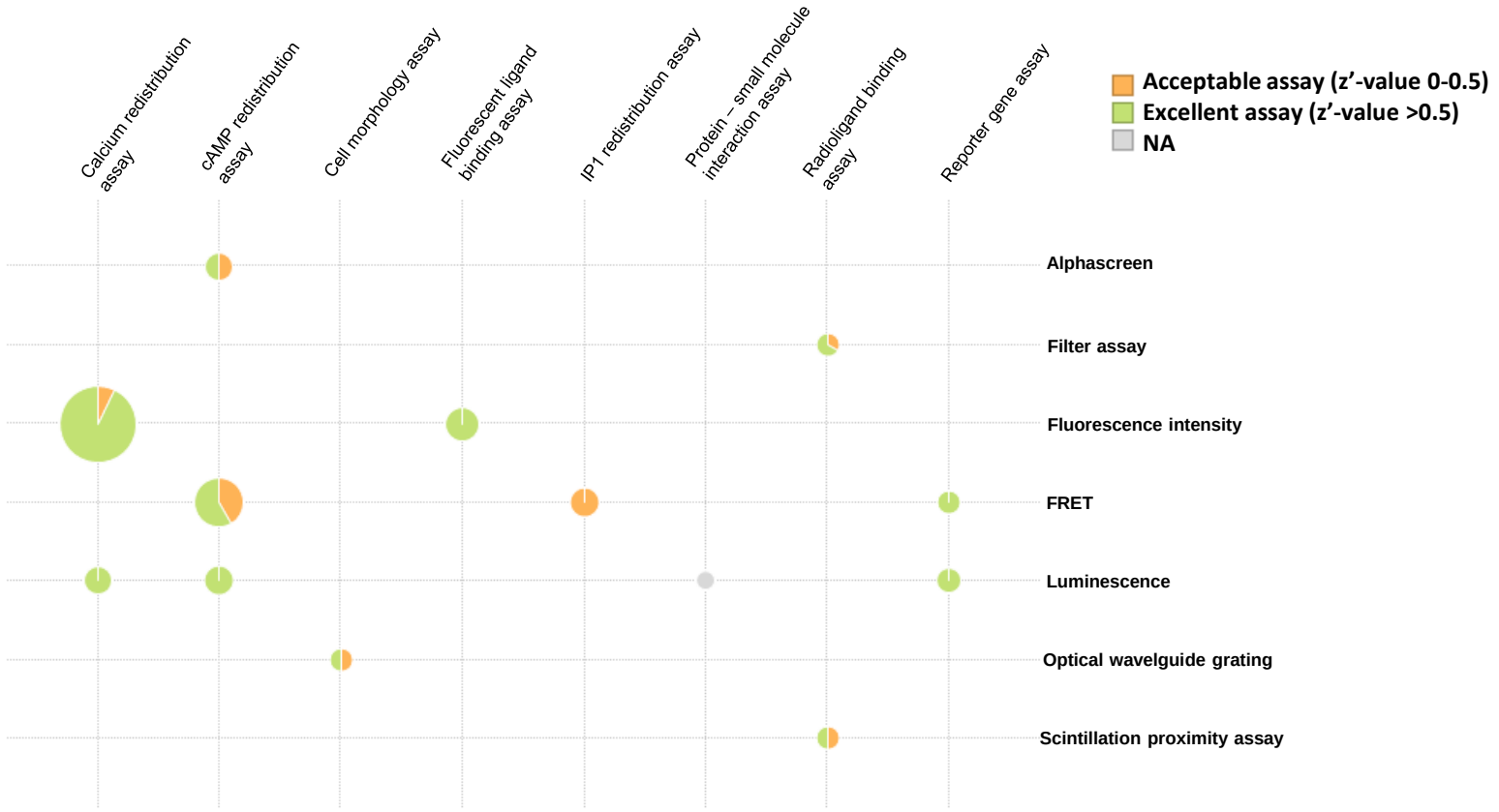
Assay design of AZ and PubChem GPCR HTS



One explanation for the low usage of cAMP redistribution method among the annotated PubChem assays could be that no class B GPCRs has been screened

Assay development support

z' value of AZ GPCR target HTS assay design and detection technology



Assay development support

Method usage of common targets between annotated AZ and PubChem HTS assays

Gene	Source	Format	Assay design	Technology
	AstraZeneca	cell membrane format	radioligand binding assay	filter assay
	AstraZeneca	cell-based format	cAMP redistribution assay	fret
XXX	PubChem	cell-based format	beta galactosidase reporter gene assay	luminescence method
	AstraZeneca	cell-based format	beta galactosidase reporter gene assay	luminescence method
XXX	PubChem	cell-based format	beta galactosidase reporter gene assay	luminescence method
	AstraZeneca	cell-based format	calcium redistribution assay	fluorescence intensity
XXX	PubChem	cell-based format	calcium redistribution assay	fluorescence intensity
	AstraZeneca	biochemical format	direct enzyme activity measurement method	fret
XXX	PubChem	biochemical format	direct enzyme activity measurement method	fluorescence intensity
	AstraZeneca	cell-based format	cAMP redistribution assay	luminescence method
	PubChem	cell-based format	luciferase reporter gene assay	luminescence method
XXX	PubChem	cell-based format	calcium redistribution assay	fluorescence intensity
	AstraZeneca	biochemical format	fluorescent ligand binding assay	fret
	PubChem	biochemical format	protein-protein interaction assay	fret
XXX	PubChem	cell-based format	protein redistribution assay	flow cytometry
	AstraZeneca	tissue-based format	direct enzyme activity measurement method	spectrophotometry method
	AstraZeneca	biochemical format	protein-small molecule interaction assay	optical waveguide grating
XXX	PubChem	biochemical format	direct enzyme activity measurement method	fluorescence intensity
	AstraZeneca	cell-based format	fluorescent ligand binding assay	fluorescence intensity
	AstraZeneca	cell-based format	calcium redistribution assay	fluorescence intensity
	AstraZeneca	cell-based format	calcium redistribution assay	luminescence method
XXX	PubChem	cell-based format	protein-small molecule interaction assay	flow cytometry
	AstraZeneca	biochemical format	fluorescent ligand binding assay	fluorescence polarization
XXX	PubChem	cell-free format	chaperone activity assay	luminescence method
	AstraZeneca	cell membrane format	radioligand binding assay	SPA

Assay development support

Comparison for a GPCR between in-house and PubChem data

AstraZeneca	**calcium redistribution**	**fluorescence intensity**	**Calcium-3 dye**	**520-545**	**FLIPR tetra**	**CHO**
PubChem	**calcium redistribution**	**fluorescence intensity**	**Fluo 8 Calcium indicator**	**515-575**	**FLIPR tetra**	**CHO**

AstraZeneca assay z'-value >0.5

24% overlap

		AstraZeneca	
		Active	Inactive
PubChem	Active	17	79
	Inactive	27	41293

		AstraZeneca	
		Retest	**Parental cell line**
PubChem	Active	15(2)	14(1)
	Inactive	9(18)	9

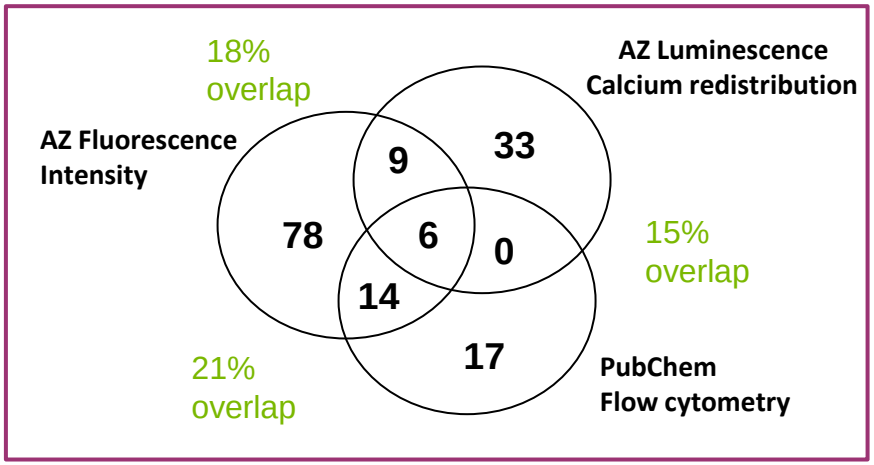
Parental cell line counter screen

		AstraZeneca	
		Active	Inactive
PubChem	Active	17	52(27)
	Inactive	27	41293(135)

Assay development support

Comparison for a GPCR between in-house and PubChem data

AstraZeneca	calcium redistribution assay	luminescence method	Aequorin		CHO
AstraZeneca	fluorescent ligand binding assay	fluorescence intensity	Alexa647	647	293
PubChem	protein-small molecule interaction	flow cytometry	FITC	515-545	U937



Frequent hitter analysis

Enzyme target using fluorescent technology

Standard frequent hitter analysis- compounds active in several unrelated assays such as fluorescent compounds, compounds forming micelles or low complexity compounds binding across target families.

- **enzyme activity assays**
- **assays detected by fluorescence**

Frequent hitter analysis using assays annotated according to BioAssay Ontology:

- ✓ **target class**
- ✓ **assay format**
- ✓ **assay design**
- ✓ **detection technology**
- ✓ **specifications like wavelength**

Frequent hitter analysis

- **Assay design: Enzyme activity**
- **Mode of action: Inhibitory**

- **Assay format: Micelle**
- **Detection technology: FRET**
- **Wavelength: ex 485, em 520 and 590 nm**

Three in-house frequent hitter reference assays were identified:

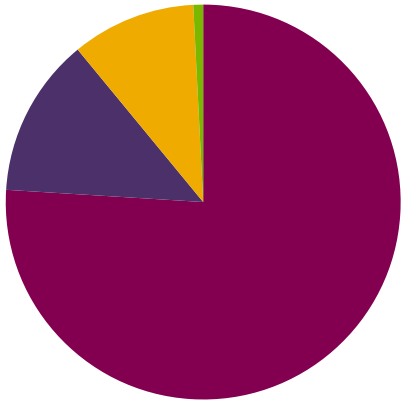
- **Assay format: Single protein**
- **Detection method: Fluorescence intensity**
- **Wavelength: ex 485, em 520/535 nm**

- **different enzyme target classes**

Frequent hitter analysis

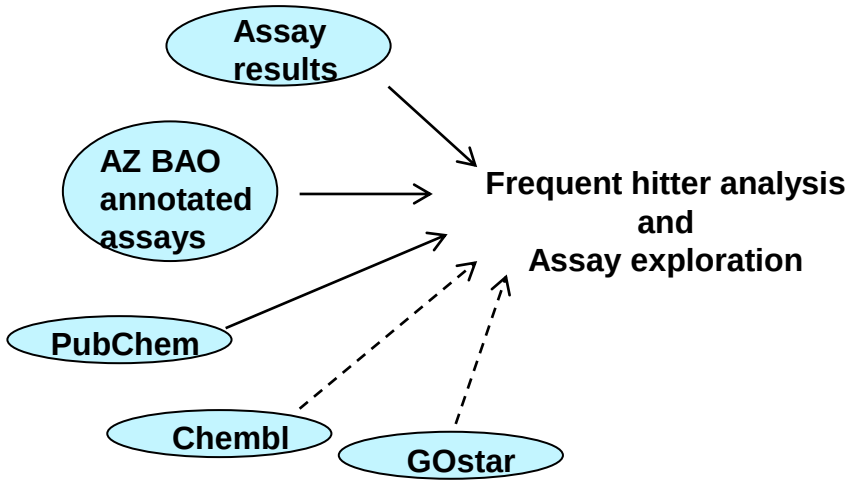
Enzyme target using fluorescent technology

Substances confirmed in concentration response assay



Frequent hitter analysis using three BioAssay ontology annotated reference assays identified almost twice as many frequent hitters as the ordinary frequent hitter analysis without the granularity of BAO.

Summary



• Detailed information of annotated HTS assays and project cascades according to BioAssay ontology classifications.

• Analysis with external assays annotated according to BioAssay Ontology from PubChem, Chembl and GOstar (Chembl and Gostar annotation needs to be done)

• Compound activity data for tool compound identification from both internal and external data

• Exploration of internal and external assays, which can be used as decision support during assay development and screening cascade design.

• Customized analysis of a subset of in-house assays defined by BioAssay Ontology classes jointly with compound activity data from from PubChem for statistical frequent hitter analysis.

Conclusions

- **The evaluation of HTS robustness and reproducibility is facilitated by the annotation of assays according to BioAssay Ontology.**

- BAO is implemented and an integral part of AZ HTS analysis strategy

- BAO facilitates identification of screening technologies used for similar targets internally and externally and the robustness of a specific assay technology for a target (sub-)class can be estimated

- BAO facilitates identification of external screening data that can confirm assay reproducibility

- BAO improves frequent hitter analysis

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