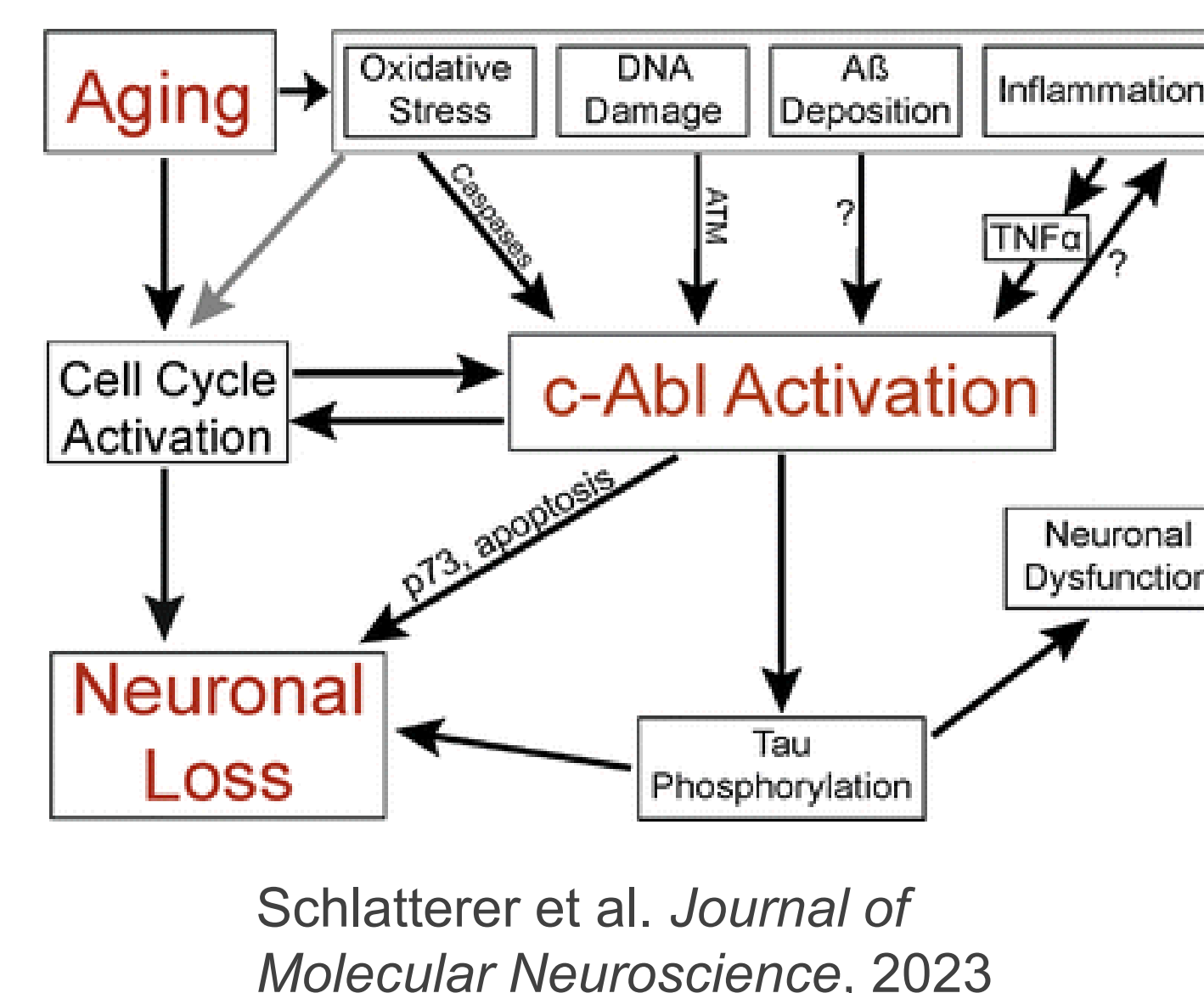
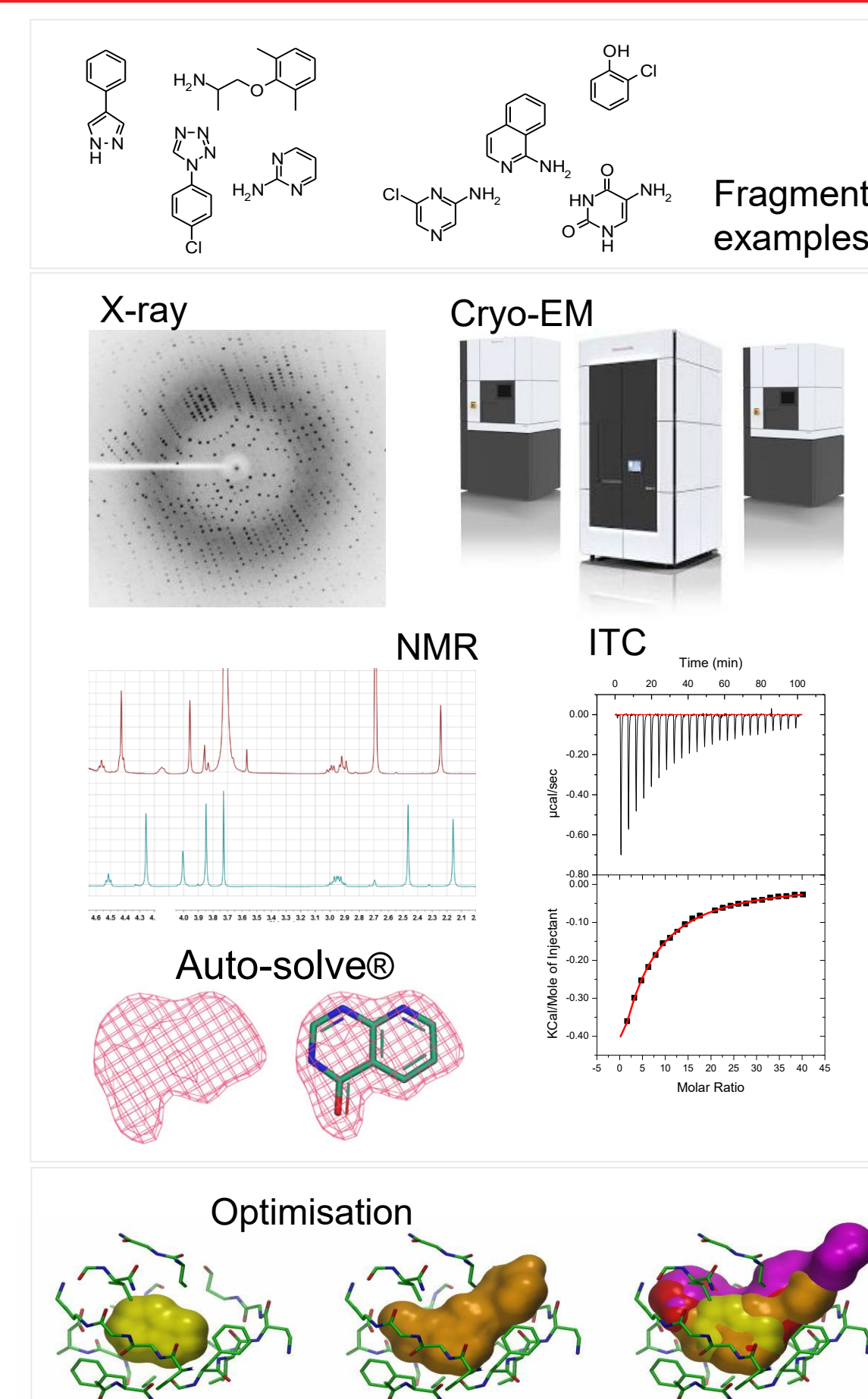
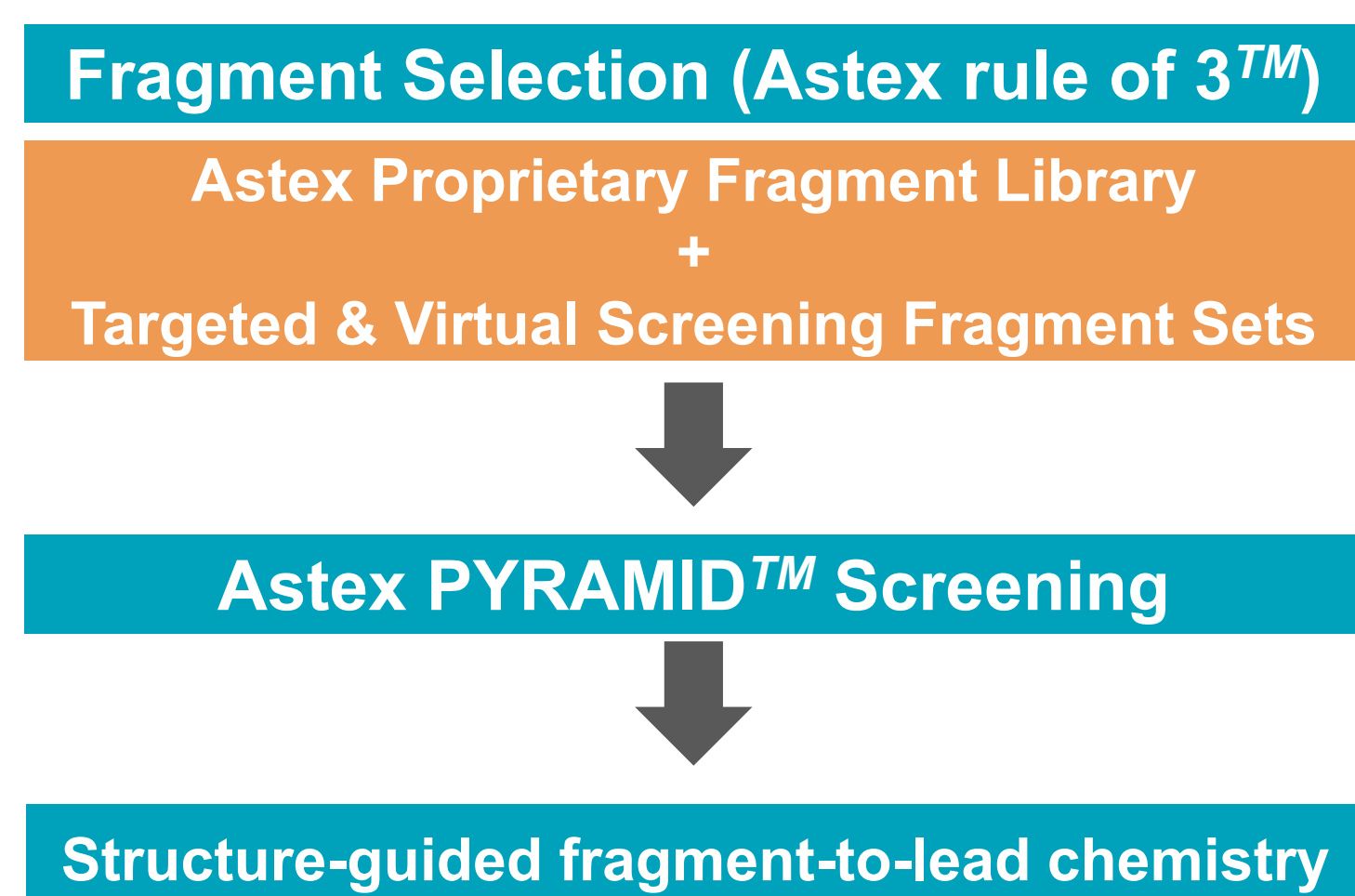


Lizzie Glennon, Anthony Dossang, Alpesh Shah, Navi Kandola, Don Murphy, Tomoko Smyth, Heather Weir, Nicola Wilsher, David Norton, Amanda Price, Nicola Wallis, Gregory A. O'Sullivan*, Elena Di Daniel, Carla F. Bento
Astex Pharmaceuticals, 436 Cambridge Science Park, Milton Road, Cambridge, CB4 0QA, United Kingdom. *Presenter

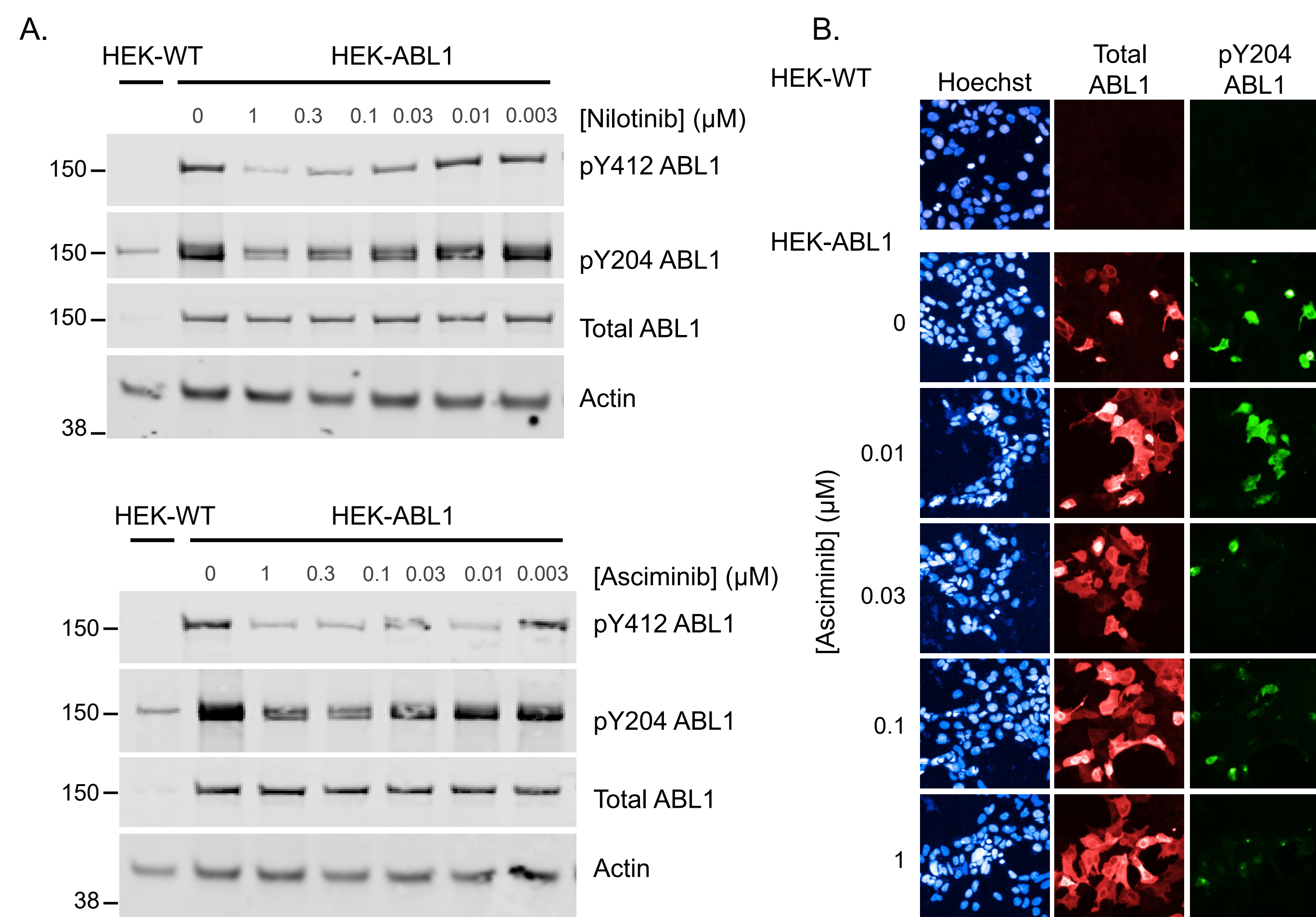
- Oxidative stress and mitochondrial dysfunction play a role in dopaminergic neuron degeneration in Parkinson's Disease (PD).
- The tyrosine-protein kinase ABL1 is activated by oxidative stress and has been shown to phosphorylate proteins involved in PD such as SNCA (alpha-synuclein), PRKN (Parkin) and ZNF746 (PARIS).
- Further, published pre-clinical *in vivo* data, involving several different ABL1 inhibitors, has shown that this pathogenic stress response can be dampened and that downstream targets such as SNCA aggregates can be reduced.
- In our efforts to develop novel proprietary ABL1 inhibitors, we have generated robust and quantitative assays to measure stress-induced autophosphorylation of ABL1 and one of its reported substrates, SNCA.
- We show that phosphorylation of tyrosine residue Y204 is also induced in response to oxidative stress and PDGFR signalling *in vitro* and reduced by treatment with ABL1 inhibitors both *in vitro* and *in vivo*. Further, a custom-made ultra-sensitive SIMOA assay successfully detected phosphorylation of Y204 in mouse brain where endogenous levels of ABL1 are known to be low.
- We also developed an assay to measure phosphorylated Y39 SNCA in HEK293 cells through the overexpression of both ABL1 and SNCA. Treatment of these cells with known orthosteric or allosteric ABL1 inhibitors reduced the number of phosphorylated Y39 SNCA aggregates.
- In conclusion, we have developed (1) cell-based assays to measure phosphorylation of ABL1 at Y204 and SNCA at Y39, and (2) an ultrasensitive assay to quantify activated/phosphorylated ABL1 in rodent brain.



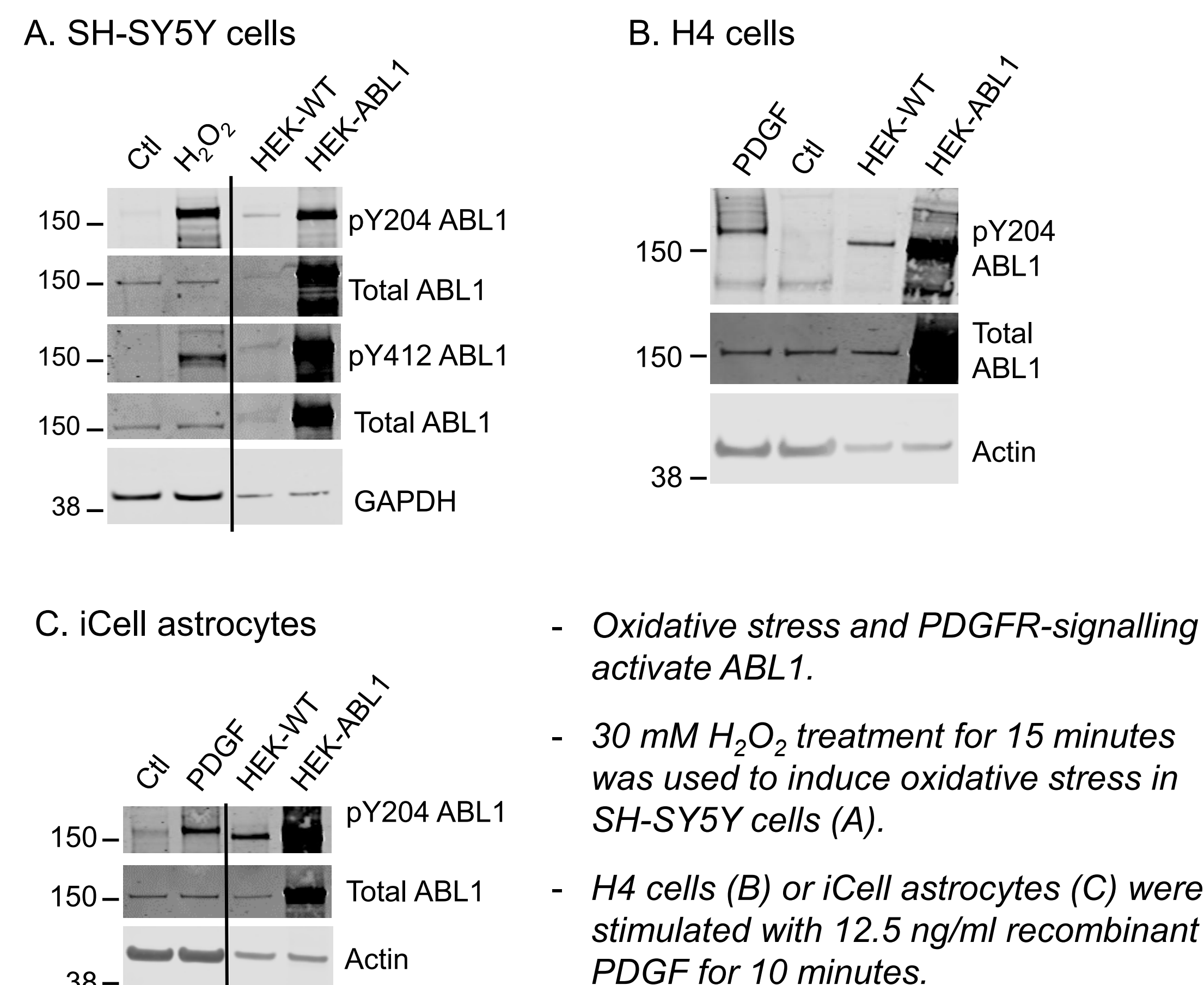
- Libraries of fragments sample chemical space more efficiently than leads or drug-sized molecules.
 - Typical properties of Astex fragments are: MW ~ 140-230 Da; non-hydrogen atoms 10-16; cLogP (lipophilicity) ~ 0.0-2.0; aqueous solubility > 5 mM in 5% DMSO; stability > 24 hours in solution.
 - Potencies of small fragments in mM range (rather than μ M range as for HTS hits) require sensitive biophysical techniques to detect interactions.
 - Fragment hits bind with good affinity for their size (high ligand efficiency / LE).
 - At Astex, we aim to have a structural-driven hypothesis for every hit allowing rapid design and optimization into larger molecules with higher affinities and desirable properties. Therefore, mM fragments are converted to nM leads with the support of structure-based drug design.
 - Fragment screens often identify novel binding sites.
 - Our proprietary FBDD platform allows exploitation of novel pharmacological profiles and more challenging targets with an emphasis on:
 - improved selectivity and potency
 - alternative mechanisms of action such as allosteric modulation and protein-protein interactions
 - improved drug-like physicochemical properties
- #### a) Fragment hit
-
- Rees et al. *Nature Reviews*



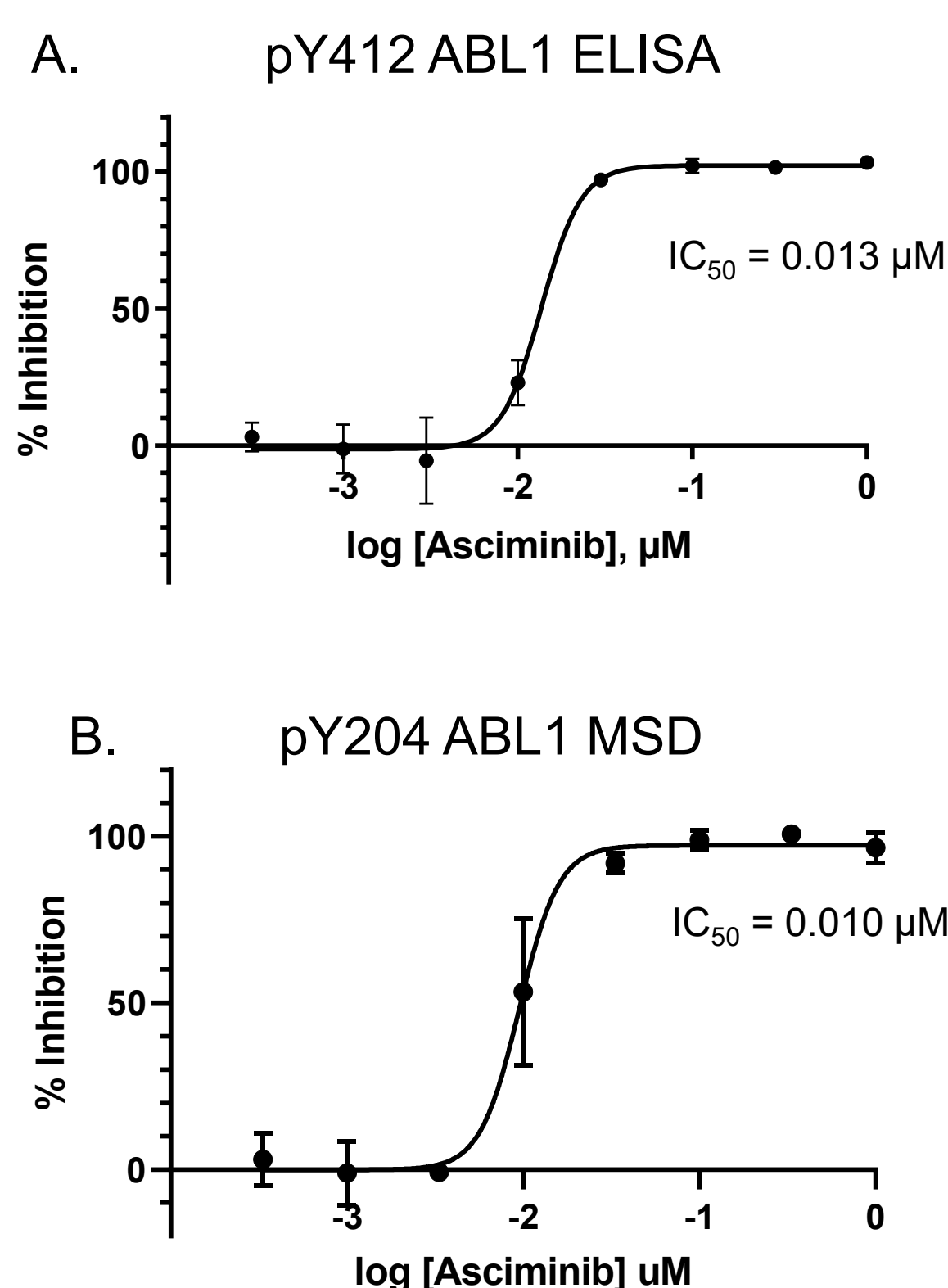
Dose-dependent reduction in ABL1 activation (pY412 or pY204) by ABL1 inhibitors in HEK293 cells overexpressing ABL1



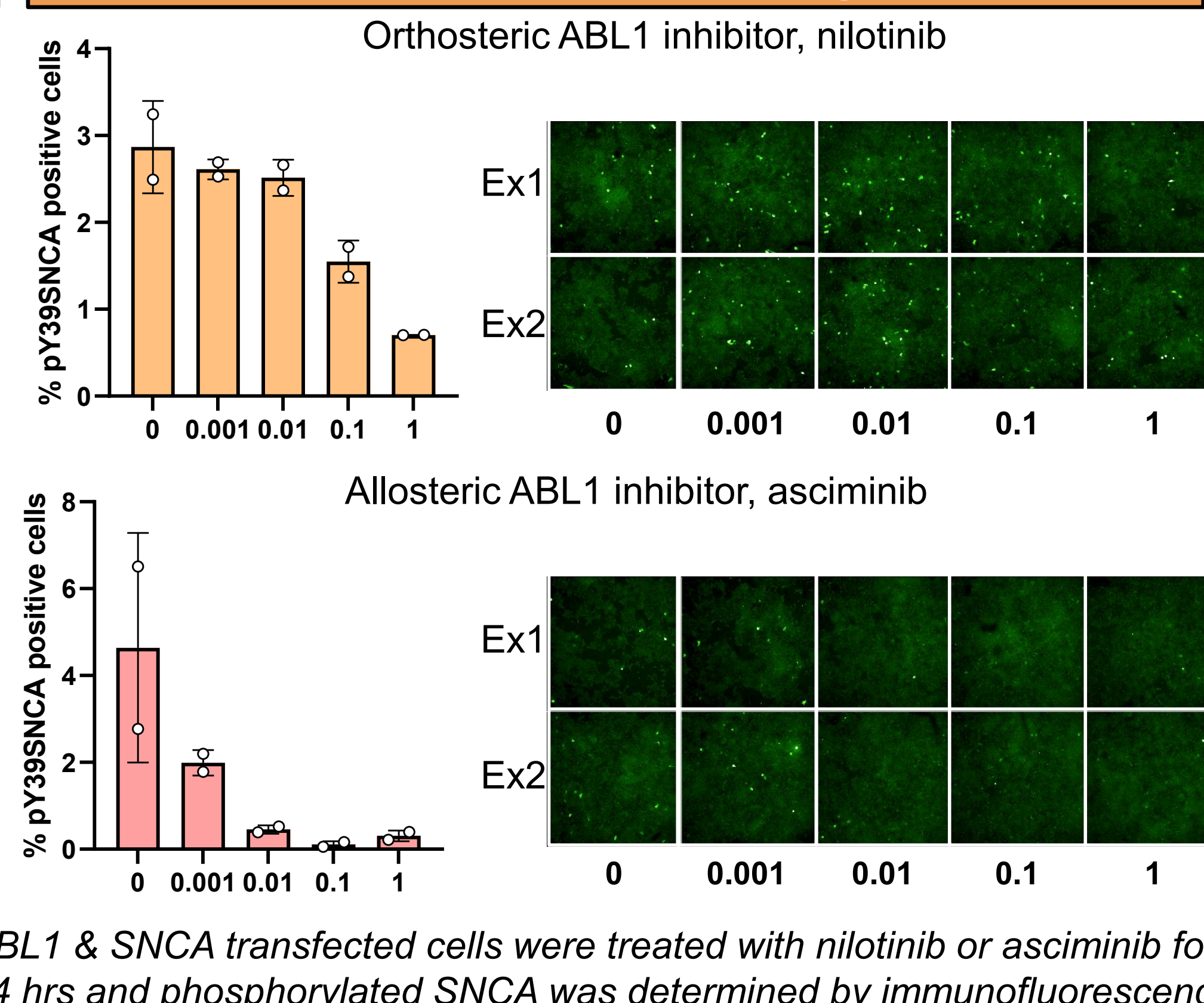
Demonstration of endogenous ABL1 activation (pY204 or pY412) after exposure to exogenous stressors in multiple cell lines



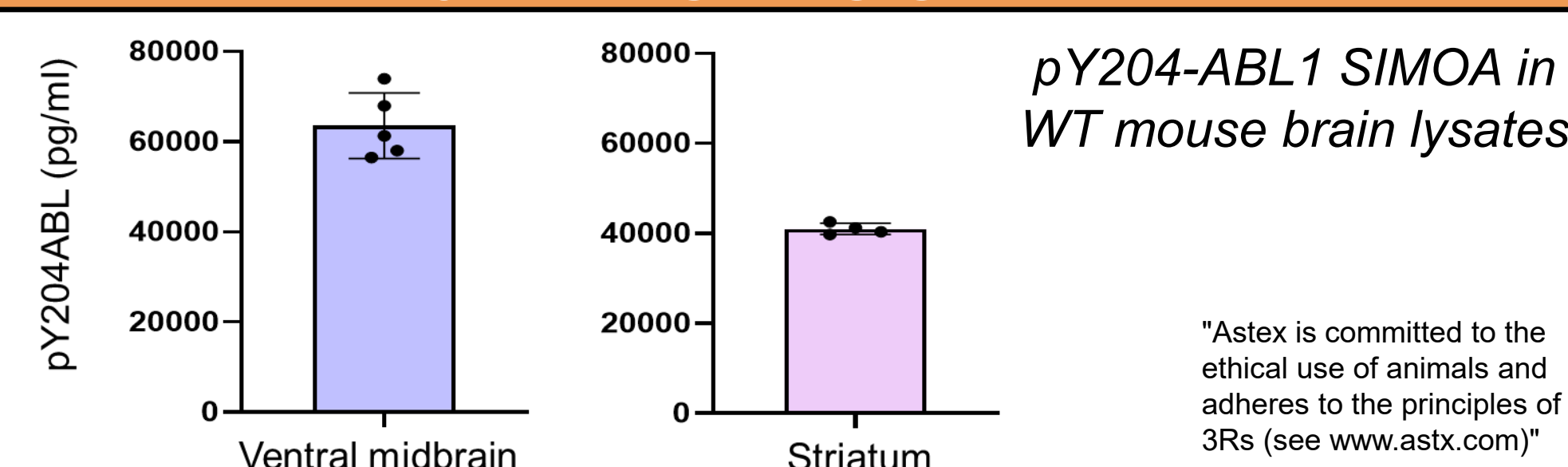
Asciminib results in a dose-dependent reduction of activated (overexpressed) ABL1 (pY412/pY204)



ABL1 inhibition has an impact on SNCA phosphorylation at residue Y39 in HEK293 overexpressing ABL1 and SNCA



**Activated ABL1 (pY204) can be detected in wild-type mouse brain;
Potential utility as a target engagement marker**



The assays described, alongside other assays implemented at Astex, have utility not only in helping us to understand mechanisms linking ABL1 and disease, but also as potential Target engagement and disease relevant pharmacodynamic marker assays. As such, these will prove useful in the evaluation of Astex proprietary ABL1 inhibitors that have been designed using our fragment- and structure-based drug discovery approaches.



astex[™]