Interrogating potential drug targets for Parkinson's disease

BIOCHEMICAL AND CELL-BASED ASSAYS FOR DOPAMINE D2 AND LRRK2.

Parkinson's disease (PD), a degenerative disorder of the central nervous system caused by a loss of dopamine-secreting neurons, results in altered neuronal activity within regions of the brain that regulate movement. Classical symptoms of the disease include resting tremors, bradykinesia, rigidity, and postural instability. Current therapies have focused on treating the symptoms of PD; however, recently identified genetic mutations may spur the development of new therapies to address the cause of the disease. This complex disease involves many cellular components and pathways, offering a variety of potential targets—including the G-protein–coupled receptor (GPCR) dopamine D2 and the serine/threonine protein kinase LRRK2 (leucine-rich repeat kinase 2)—for drug therapeutics (Figure 1). Invitrogen offers biochemical and cell-based assays for studying these key targets within the pathways regulating PD. Alternatively, you can outsource your screening and profiling projects to Invitrogen's SelectScreen® Service.

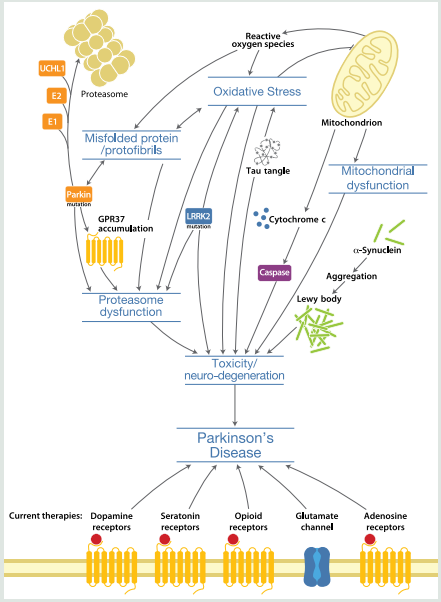

Figure 1. Schematic of cellular and pathway components involved in Parkinson's disease, along with current therapy approaches.

Measuring dopamine D2 activation using GeneBLAzer® technology

The first-line treatment for PD is the use of dopamine agonists, many of which act through the dopamine D2 GPCR. Invitrogen has developed a GeneBLAzer® cell-based assay to monitor activation of this GPCR via a beta-lactamase reporter technology. GeneBLAzer® technology employs a membrane-permeant fluorescence resonance energy transfer (FRET)-based substrate that allows detection of a functional response in live cells. The dual-emission wavelength readout significantly reduces experimental variables. To aid in the identification of possible

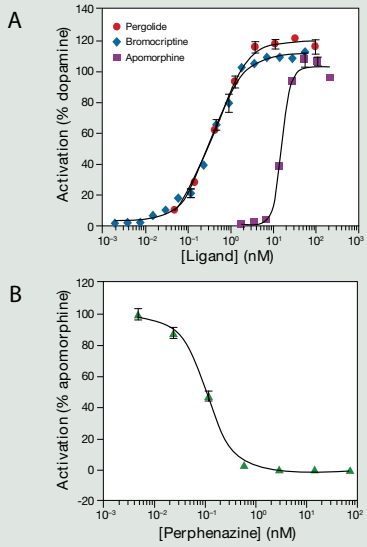

Figure 2. Measuring dopamine D2 activation or inhibition using GeneBLAzer® technology. (**A**) GeneBLAzer® D2-Gqo5-NFAT-*bla* CHO-K1 (Cat. No. K1708) cells were activated by incubation with serial dilutions of pergolide, bromocriptine, or apomorphine and then loaded with LiveBLAzer™-FRET B/G Substrate (Cat. No. K1095). (**B**) Inhibition of apomorphine-induced activation by perphenazine. GeneBLAzer® D2-Gqo5-NFAT-*bla* CHO-K1 cells were incubated with serial dilutions of perphenazine prior to stimulation with 18 nM apomorphine. Cells were then loaded with LiveBLAzer™-FRET B/G Substrate. Fluorescence emission ratios were normalized such that 0% is equivalent to the absence of stimulant while 100% equals that observed with (**A**) 1 µM dopamine or (**B**) 18 nM apomorphine.

agonists of the dopamine receptor, the GeneBLAzer® D2-Gqo5-NFAT-*bla* CHO-K1 cell line was developed to monitor activation of the D2 GPCR via a beta-lactamase reporter gene. The cell line contains the stably integrated human dopamine receptor 2 (D2), a beta-lactamase (*bla*) reporter gene under control of the nuclear factor of activated T cells response element (NFAT), as well as the chimeric G-protein Gqo5 in the CHO-K1 cell line. The results show that D2 receptor agonism can be quantified for the Parkinson's disease drugs bromocriptine, pergolide, and apomorphine; furthermore, D2 receptor antagonism was readily quantified using apomorphine as the agonist (Figure 2).

Alternatively, this cell line may be used to determine the specificity of receptor agonists and/or antagonists. The SelectScreen® Cell-Based GPCR Profiling Service allows you to not only identify new agonists and/or antagonists for the D2 receptor, but also determine the selectivity of your compounds for the receptor by profiling against a panel of any of our 132 available GPCR cell lines.

LanthaScreen® TR-FRET assay for LRRK2

Mutations in the kinase-encoding gene LRRK2 have been identified in association with PD [1]. At least 20 such mutations have been discovered, scattered across the large, multidomain protein. The most prevalent mutation, G2019S, has been demonstrated to increase the kinase activity of LRRK2. Discovering an LRRK2 inhibitor could lead to new therapeutic agents for treating the disease. Invitrogen has developed a collection of high-throughput screening tools for wild-type LRRK2 and the LRRK2 G2019S mutant. We have generated highly purified and active LRRK2 enzymes and the kinase-dead mutant LRRK2 D1994A (Figure 3), which can be used to screen potential inhibitors in a fully validated LanthaScreen® TR-FRET kinase assay. This assay provides sensitive and specific detection of LRRK2 kinase activity using a peptide substrate derived from ezrin/radixin/moesin (ERM) labeled with fluorescein. Phosphorylation of the peptide recruits a terbium-labeled phosphospecific antibody, resulting in FRET. Due to the TR-FRET readout, the assay is highly resistant to compound interference and is also suitable for evaluating inhibitor potencies (Figure 4).

Let us enable your search for potential mediators of LRRK2 activation by screening your compound libraries using our SelectScreen® Library Screening Service. We can also profile your lead candidates using the SelectScreen® Biochemical Kinase Profiling Service.

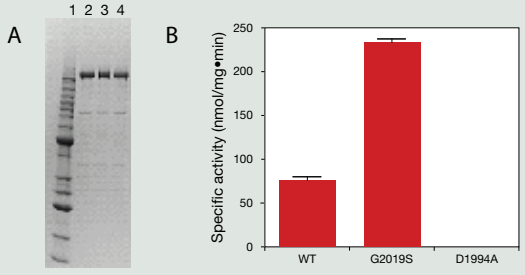

Figure 3. Analysis of wild-type LRRK2 and various LRRK2 mutants. **(A)** Purity was determined by SDS-PAGE. Lanes contain BenchMark™ Ladder (lane 1), and wild-type (Cat. No. PV4873), G2019S (Cat. No. PV4881), and D1994A (inactive) LRRK2 variants (lanes 2–4, respectively). **(B)** Activity of each LRRK2 variant was determined using a radiometric assay. LRRK2 D1994A shows no activity using this method, confirming that the kinase is inactive.

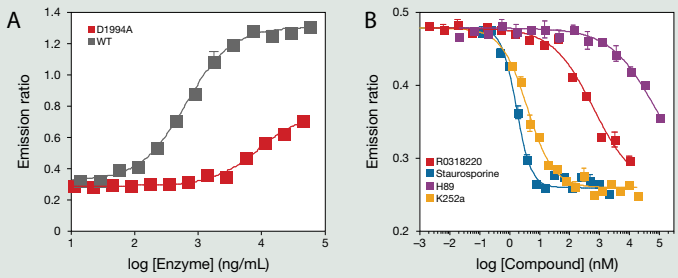

Figure 4. Validation of the LanthaScreen® assay for LRRK2. **(A)** Differentiation of response between wild-type and kinase-dead LRRK2, which was not observed with other substrates. Assay components used were the LanthaScreen® Tb-anti-ERM (pLRRKtide) Antibody (Cat. No. PV4899) and the Fluorescein-ERM (LRRKtide) Substrate (Cat. No. PV4901). **(B)** Inhibition of LRRK2 with small-molecule kinase inhibitors using the LanthaScreen® format.

Tools for studying potential PD drug targets

Take advantage of Invitrogen's biochemical and cell-based assays for dopamine D2 and LRRK2, and rely on Invitrogen's SelectScreen® Service for outsourcing your screening and profiling projects. Learn more about these assays and services at **www.invitrogen.com/bp60**. ■

Reference

1. Cookson M et al. (2007) *J Neurosci* 27:11865–11868.

Product	Quantity	Cat. No.
GeneBLAzer® D2-Gqo5-NFAT-*bla* CHO-K1	2 x 10^6 cells	K1708
LiveBLAzer™-FRET B/G Substrate	200 µg	K1095
LanthaScreen® Tb-anti-ERM (pLRRKtide) Antibody	25 µg	PV4899
Fluorescein-ERM (LRRKtide)	1 mg	PV4901
LRRK2 recombinant protein	10 µg	PV4873
LRRK2 G2019S recombinant protein	10 µg	PV4881