Selective Detection of LRRK2 GTPase Activity with the Transcreener ® GDP Assay

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Abstract

LRRK2 is a dual function protein with separate catalytic domains for kinase and GTPase activity, and there is compelling genetic evidence implicating the GTPase domain in Parkinson's disease. The activity of the GTPase domain is much lower than the kinase domain, which presents a challenge for HTS assay development. Here we show that the Transcreener GDP Assay was sensitive enough to detect GDP formation by an expressed LRRK2 fragment (aa 970-2527) and full length protein (made available through the Life Technologies Early Access program). We verified, using mutant forms of the protein, that the kinase domain was not contributing to GDP formation. We have also identified conditions for more sensitive LRRK2 detection with either form of the enzyme utilizing 1 µM GTP. The mix-and-read Transcreener GDP Assay is the ideal high throughput screening tool for elucidating the function of full length and mutant forms of LRRK2 , because it is highly amendable to automation, provides far red fluorescence polarization detection to minimize compound interference, and is sensitive enough for monitoring initial rates of catalysis.

LRRK2's GTPase Domain is an Important Target for Drug Discovery

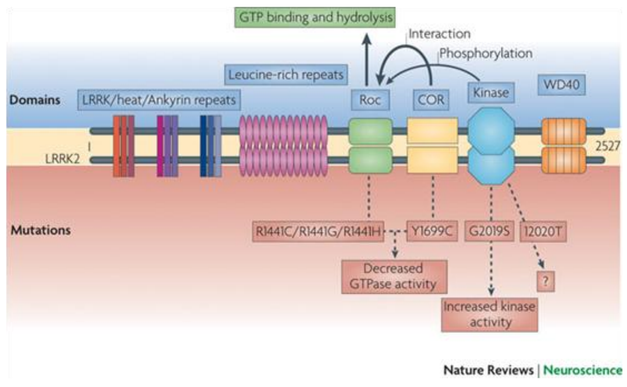

Figure 1. LRRK2 protein structure/function and location of key pathogenic mutations. LRRK2 is a large , dimeric protein with protein interaction domains surrounding a catalytic core containing separate kinase and GTPase domains. Of the many LRRK2 mutations with putative links to PD, five have been unequivocally linked to disease based on disease segregation in large families and functional studies: G2019S and I2020T are activating mutations in the kinase domain, R1441C and R1441G are in the GTPase domain and Y1669C is in the adjacent COR domain; all three cause decreased GTPase activity and increased kinase activity.

The Transcreener GDP Assay is a Versatile Assay for GDP Detection

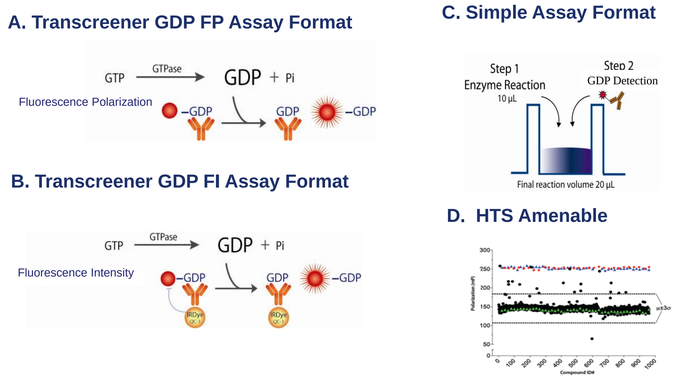

Figure 2. Transcreener GDP assay is an universal assay for GDP detection. GTPase activity can be directly detected with the Transcreener GDP monoclonal antibody. As GDP is produced it displaces a fluorescently labeled tracer which can be detected in either **A)** Fluorescent Polarization(FP) or **B)** Fluorescent Intensity(FI) format. The utilization of a far red tracer decreases compound interference.**C)** Both assays are a simple mix-and-read format with overnight signal stability. **D)** Suitability for HTS is shown by data from a pilot screen of RGS4 in combination with $G\alpha_{i1}$ (R178M/A326S) which resulted in an assay window of 120mP and a Z' of 0.83.

LRRK2 GTPase Activity is Dependent on its GTPase Domain

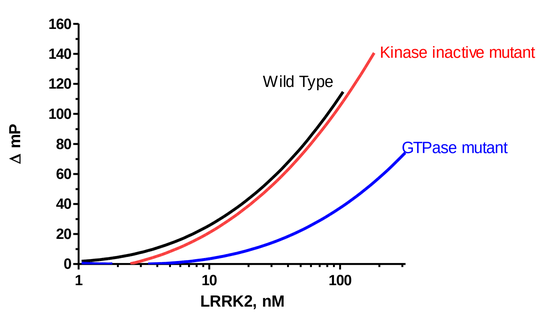

Figure 3. LRRK2 GTPase activity is dependent on its GTPase domain: Similar to many regulatory GTPases, unstimulated LRRK2 GTPase activity is very low, and the much higher kinase activity (>1,000x) could potentially contribute to non-specific hydrolysis of GTP. To address this question, we tested a kinase-dead LRRK2 protein (D1994A) and found that the rate of GDP formation was identical to the wild-type protein (0.03 nmol/min/mg). This result strongly suggests that the kinase domain is not contributing significantly to GDP formation. In the same experiment, we tested the GTPase deficient (R1441C) LRRK2 protein and showed that it had about 20% the GTPase activity of the wild type protein.

Full Length, Wild Type LRRK2 GTPase activity is GTP Dependent

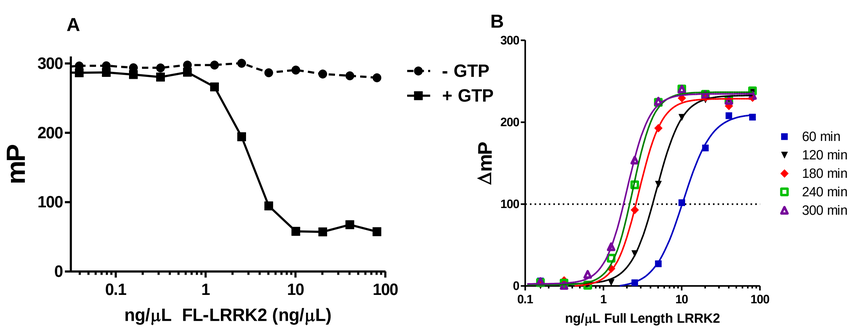

Figure 4. Full Length LRRK2 Enzyme Titration and Time Course: Enzyme titrations were performed with Full-Length LRRK2 provided by Life Technologies. A) Polarization values for an 180 minute time point were plotted in the presence(solid line) and absence(dashed line) of GTP, demonstratng that the reaction is GTP dependent. B) A time course of the LRRK2 titration is shown from 60-300 minutes($_{\Delta}$MP = mP $_{\text{No LRRK2}}$- mP $_{\text{LRRK2}}$). Unless specified in legend, assay conditions are 20mM Tris, pH7.5, 1mM EDTA, 10 mM MgCl2, 0.01% Triton, 10 µM GTP, 10 µg/mL GDP Ab, and 4nM GDP Tracer.
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Optimization of LRRK2 GTPase Reaction Conditions

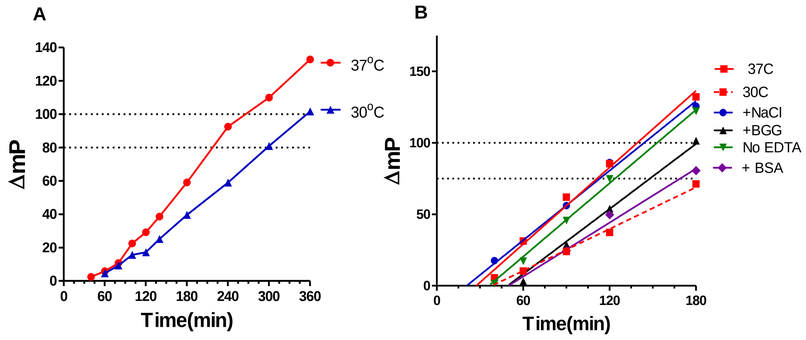

Figure 5. Optimization of LRRK2 GTPase reaction: A) A time courses using 2.0 ng/µL full length LRRK2 GTPase shows increases activity at 37ºC. B) Various factors were tested for their effect on the LRRK2 GTPase reaction. Only temperature had a significant effect. Omission of EDTA actually decreased the assay window.

Determination of LRRK2 GTPase Rates with Multiple Substrate Concentrations to Minimize Enzyme Usage

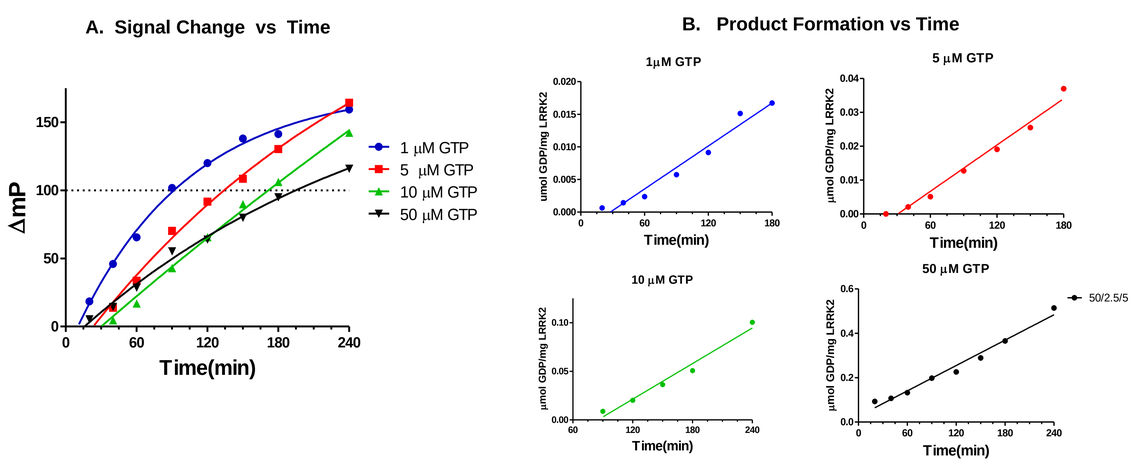

Figure 6. LRRK2 Substrate Optimization and rate determinations A) Time courses were run at varying GTP concentrations with 2.5 ng/µL full length LRRK2 (ΔMP = mP $_{\text{No LRRK2}}$- mP $_{\text{LRRK2}}$). Note that the best signal was obtained at the lowest GTP concentration (1 µM) **B)** Polarization values were converted into GDP formation based on standard curves. **C)** Rates from **B** were plotted vs GTP concentrations.

Conclusions

- Transcreener GDP assay is a simple, universal GDP detection assay available in multiple formats and is HTS amenable.
- LRRK2 GTPase activity can be detected with the Transcreener GDP assay.
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- LRRK2 kinase domain is not contributing significantly to GDP formation.
- LRRK2 GTPase activity is GTP dependent and requires its GTPase domain.
- GTPase activity can be detected with full length and mutant LRRK2 constructs.
- The assay format is very versatile, allowing for easy optimization, rate determinations and produces meaningful data.

Acknowledgements

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