Targeting the cGAS-STING Pathway Using a Homogenous, HTS Compatible Transcreener® cGAS Assay

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Overview

Cyclic GMP-AMP synthase (cGAS) is a recently discovered enzyme that acts as a foreign DNA sensor to elicit an immune response to pathogens via activation of the STING (stimulator of interferon genes) receptor. Shortly after its discovery in 2013, aberrant activation of cGAS by self-DNA was shown to underlie debilitating and sometimes fatal autoimmune diseases, such as systemic lupus erythematosus (SLE) and Aicardi–Goutieres Syndrome (AGS). Knockout studies in animal models have clearly indicated that inhibiting cGAS is a promising approach for therapeutic intervention. However, there are no HTS-compatible assay methods for measuring cGAS enzyme activity or for monitoring cGAMP in cell and tissue samples. To enable HTS efforts targeting cGAS, we developed Transcreener-based assays for cGAMP detection; i.e., homogenous, competitive immunoassays with fluorescence polarization (FP) and time resolved Förster resonance energy transfer (TR-FRET) signals. The key assay reagents are antibodies that selectively bind cGAMP in the presence of excess ATP and GTP and tracers that are displaced by cGAMP. We validated both assays with full length human cGAS and performed pilot screens for cGAS inhibitors with the FP assay. Similar to other Transcreener assays, the cGAMP assays have the performance characteristics needed for small molecule screening including sensitivity, robustness (good dynamic range, minimal signal variation), and low levels of compound interference. The availability of robust, HTS compatible assays for measuring cGAS enzyme activity will accelerate efforts to target the cGAS-STING pathway for autoimmune diseases.

The cGAS-cGAMP-STING Pathway Activates the Immune System in Response to Cytosolic DNA

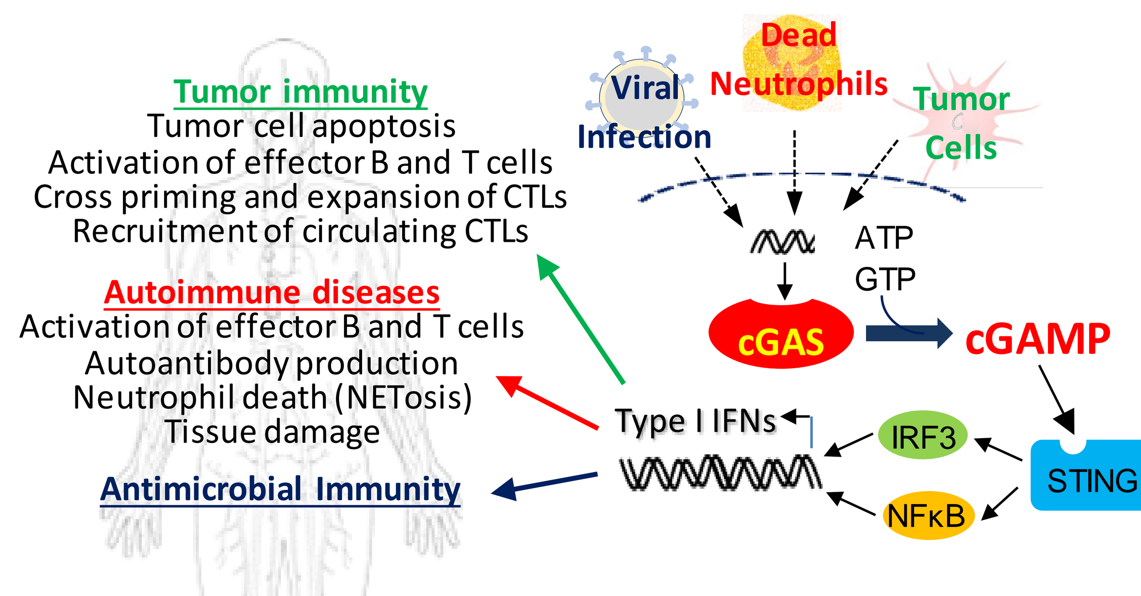


Figure 1. IFN-driven immune responses triggered by cGAS/STING are critical for protection against many types of microbial pathogens and for tumor cell-specific T cell responses in cancer, but activation by self-nucleic acids can contribute to serious autoimmune diseases such as lupus.

Mix and Read HTS Enzymatic Assay for cGAS Based on Immunodetection of cGAMP

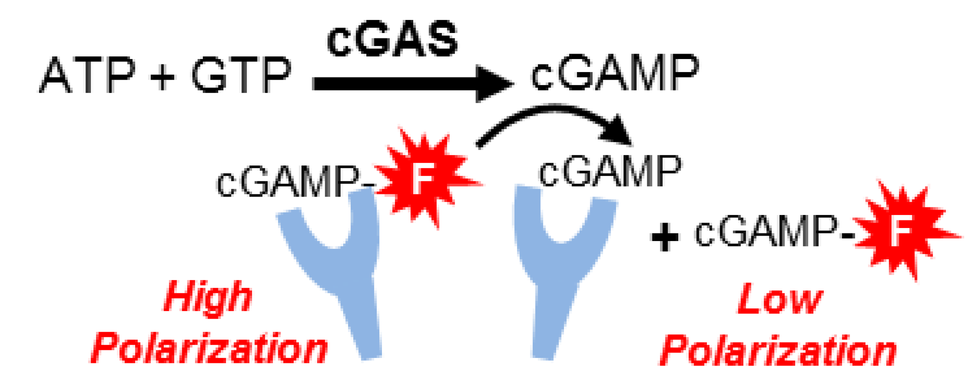


Figure 2. Transcreener cGAS Assay principle: in the competitive fluorescence polarization (FP) immunoassay for cGAMP, enzymatically generated cGAMP displaces a fluorescent tracer from mAb causing a decrease in its polarization.

Nanomolar Sensitivity and Outstanding Selectivity

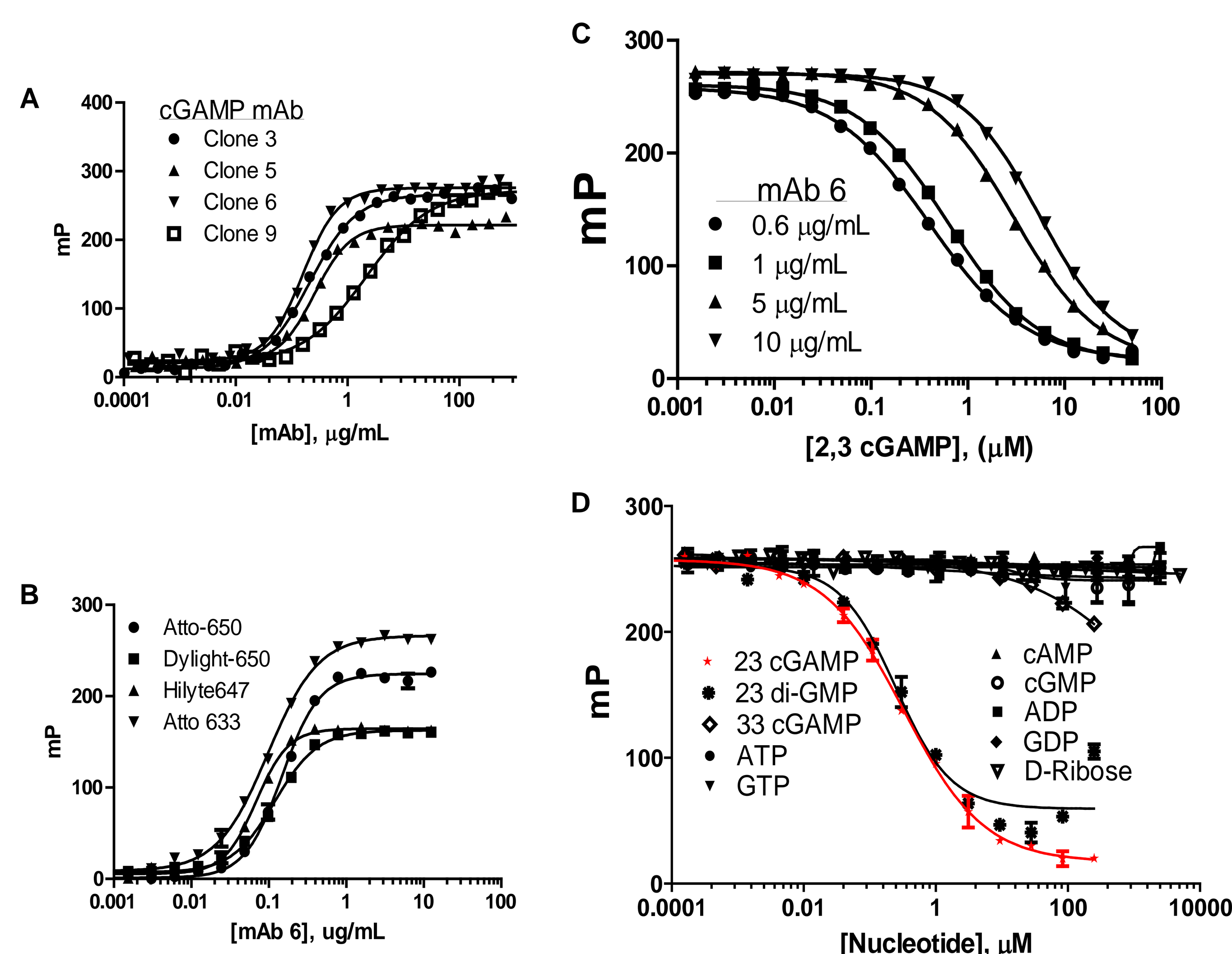


Figure 3. A. Binding curves for cGAMP mAbs and cGAMP-Atto 633 tracer. Similar analyses were carried out with several tracers: cGAMP mAb #6 was chosen for further assay development. **B. Binding curves for cGAMP mAb #6 and representative cGAMP-Fluor tracers:** Fluors were attached to cGAMP via two carbon linkers; the Atto 633 tracer was used chosen for further assay development. **C. Competition curves** indicate displacement of tracer by cGAMP and show dependence of dynamic range on mAb concentration. **D. Specificity of mAb**: Competition binding curves show outstanding selectivity for cGAMP vs. cGAS substrates, ATP and GTP, as well as related molecules.

Validation for Detection of cGAS Initial Velocity

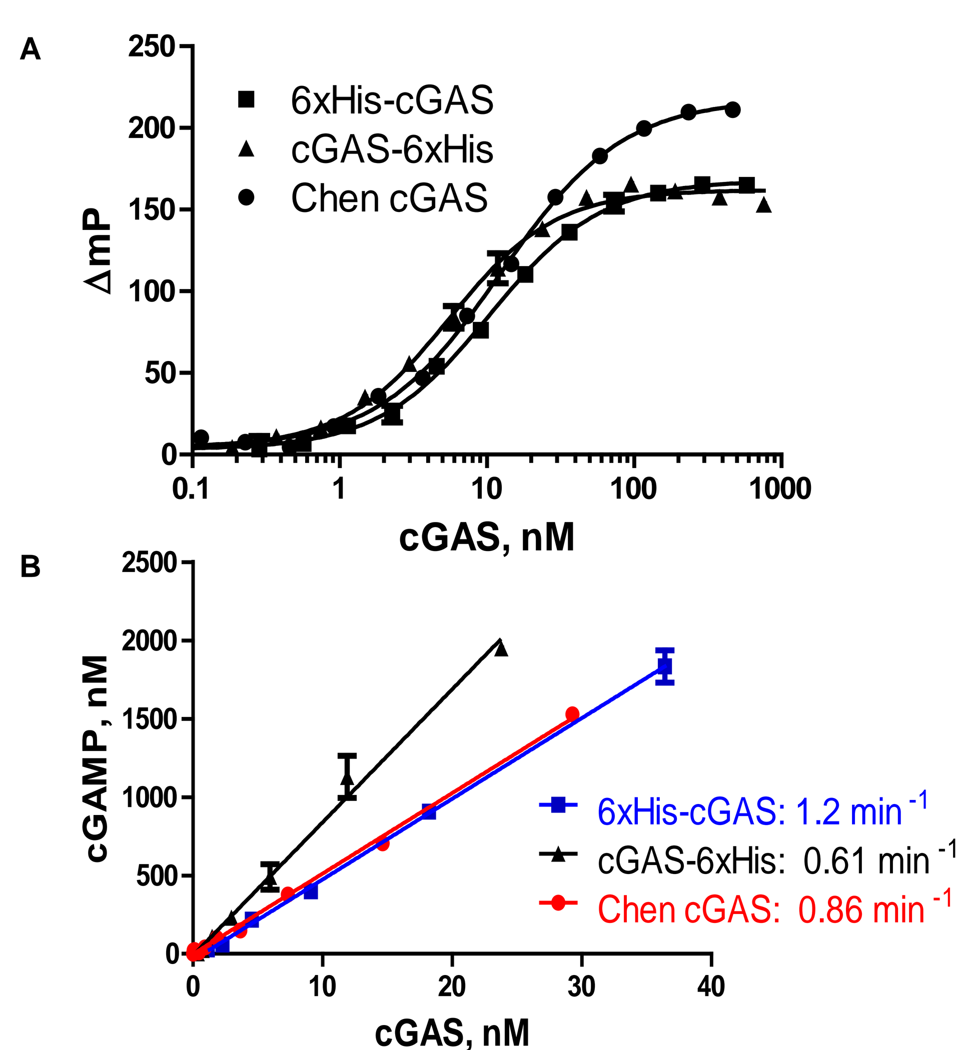


Figure 4. A. Detection of purified, full-length human cGAS. cGAS enzyme reactions contained 100 µM ATP and GTP, 62.5 nM 45 bp ISD DNA, 60 min reactions. N- and C-terminal His-tagged cGAS was produced at BBL; 6xHis-cGAS was also generously supplied by Z. Chen (UTSW Medical Center). **B. Linear response**: Polarization data from A. was converted to cGAMP using a standard curve.

Substrate and DNA Dependence

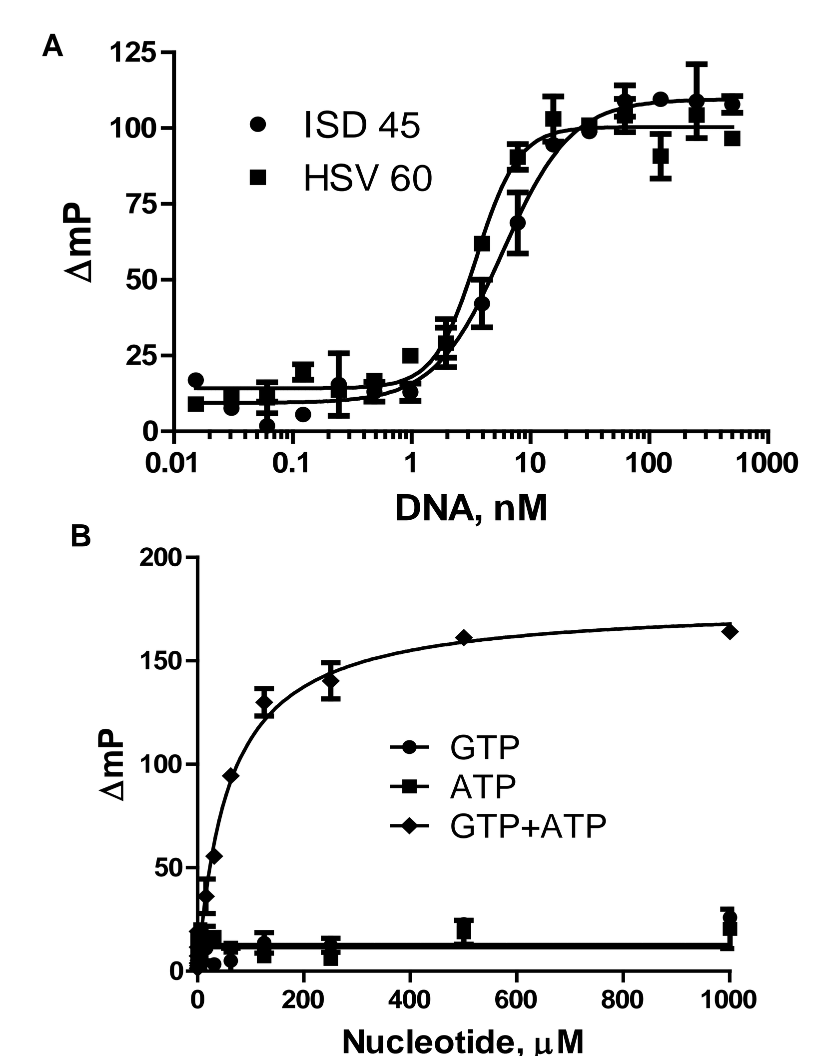
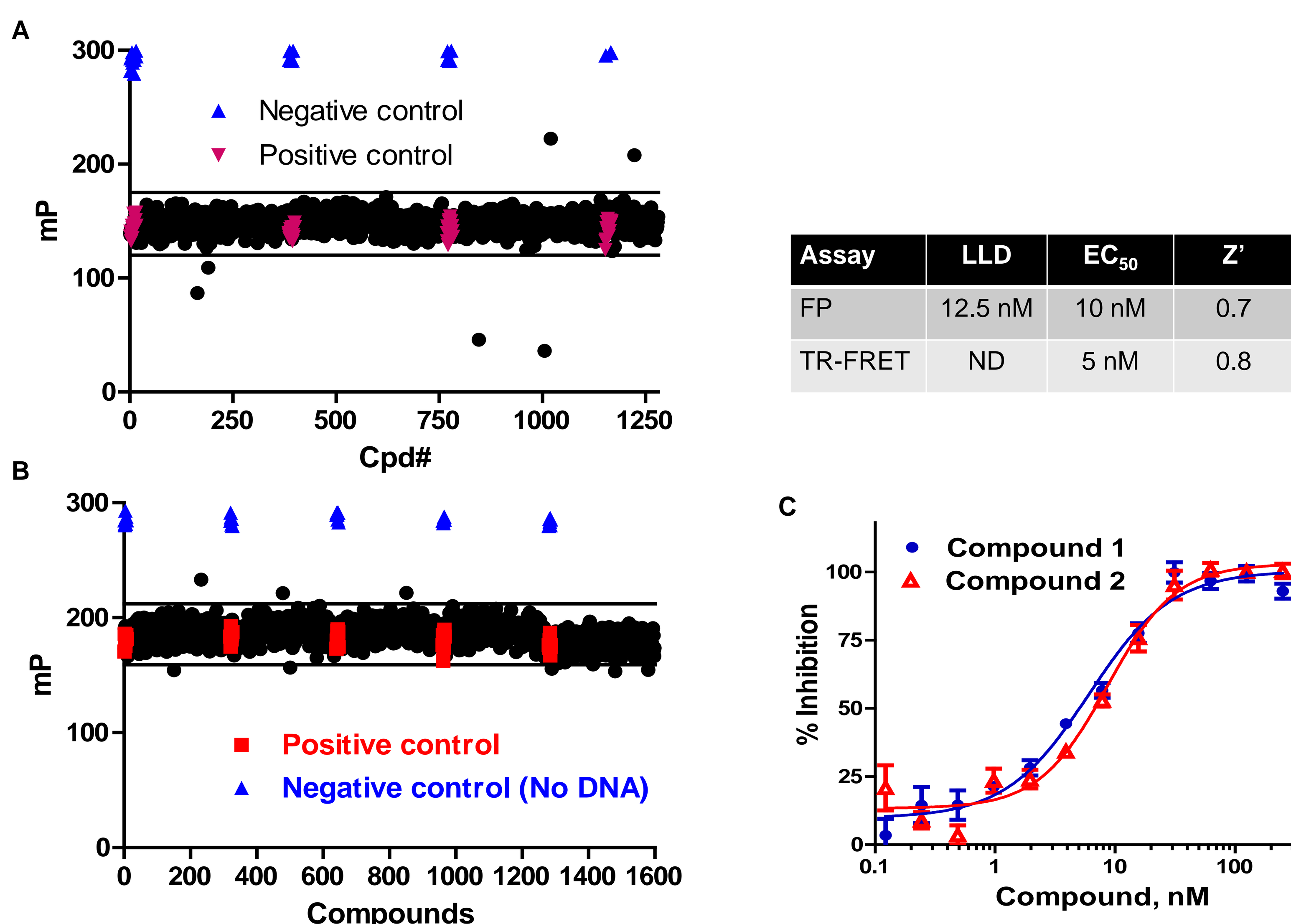


Figure 5. A. DNA dependence cGAS reactions as in Figure 4, with cGAS-6xHis at 10 nM; half maximal responses of 3.5 and 5.9 nM for HSV 60 and ISD 45, respectively. **B. ATP and GTP dependence:** ATP and GTP were titrated separately and simultaneously; cGAS reactions as in Figure 4, with cGAS-6xHis at 10 nM.

Pilot Screens for cGAS Inhibitors



Assay	LLD	EC_{50}	Z'
FP	12.5 nM	10 nM	0.7
TR-FRET	ND	5 nM	0.8

Figure 6A. Interference screen: The LOPAC library (1280 compounds) was used at 10 µM final concentration. Wells contained all cGAS enzyme reaction components except the cGAS enzyme; positive controls contained 1 µM cGAMP, negative controls contained no cGAMP. **B. Pilot screen with 1600 diversity compounds (Life Chemicals):** cGAS was used at 10 nM, compounds were at 10 µM; 60 min reaction; negative controls lacked dsDNA (required for cGAS activation); Z = 0.62, Z' = 0.7. **C. Dose response for cGAS inhibition by two hits from pilot screen shown in B**

Transcreener TR-FRET cGAS Assay

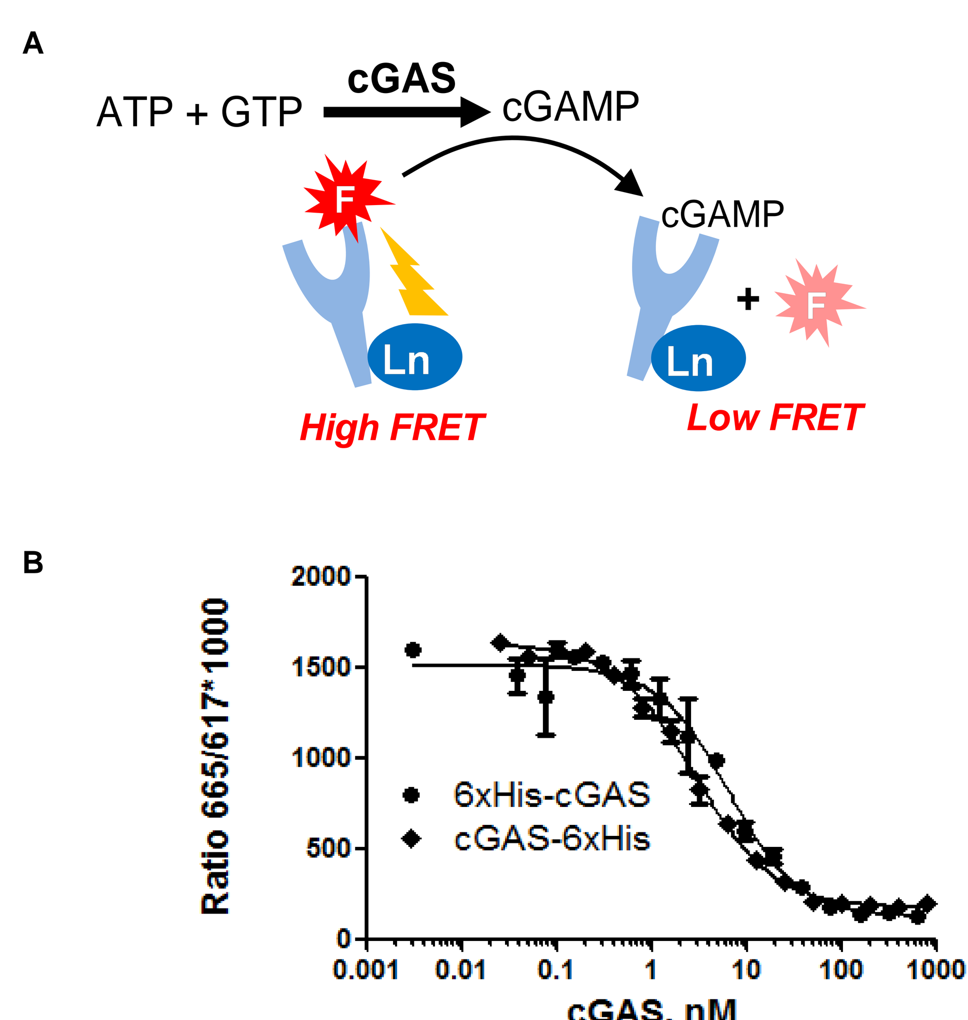


Figure 7A. Assay principle: enzymatically generated cGAMP displaces a fluorescent tracer from the lanthanide-labeled mAb causing a decrease in the TR-FRET signal. **B. cGAS enzyme titration using N- and C-terminal His tagged proteins.** As with the FP assay (Fig. 4B), conversion of FRET signal to cGAMP formation yielded a linear response (data not shown).

Conclusions

- *Competitive immunoassays for cGAMP with FP and TR-FRET readouts were developed that allowed practical detection of cGAMP over a concentration range of 0.1 to 50 µM.*
- *The assays allow detection of cGAS at low nanomolar concentrations with Z' values of 0.7 or greater.*
- *Pilot screens indicated acceptable levels of compound interference (< 0.4%) and validated the assay for discovery of cGAS inhibitors.*
- *The Transcreener cGAS Assays will accelerate efforts to test small molecule cGAS modulators for treatment of autoimmune diseases such as SLE and AGS.*

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