Kinetic Monitoring of Compound Dissociation from Wild-type and Mutant Kinase in Living Cells with NanoBRET™

Takeomi Inoue[1], Aki Emi[1], Deborah J. Moshinsky[2], James D. Vasta[3], Yugo Narumi[1], Yuko Uno[1], Ryoko Nakai[1], Masaaki Sawa[1], Matthew B. Robers[3], Yusuke Kawase[1]

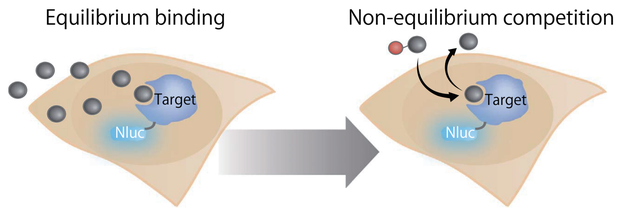
[1] Carna Biosciences, Inc., BMA, 1-5-5, Minatojima-Minamimachi, Chuo-ku, Kobe 650-0047, Japan
[2] CarnaBio USA, Inc., 209 West Central Street, Suite 307, Natick, MA 01760, USA
[3] Promega Corporation, 2800 Woods Hollow Road, Fitchburg, WI 53711, USA

Abstract: It has been recognized that drug-target residence time, in addition to affinity, is a critical parameter influencing drug efficacy. In this study, we illustrate the utility of the NanoBRET™ Target Engagement Assay for interrogating the kinetics of compound binding in living cells. In a case study of FGFR2, it was revealed that drug-target residence time of a single compound varied markedly between wild-type and mutant kinases, and among mutant kinases. These results stress the importance of evaluating the residence time of drugs against a broad range of kinases including mutants to gain insight into the kinetic selectivity of compounds.

Background:

- Drug-target residence time has been traditionally investigated using isolated kinase, but not under physiological condition such as the intracellular environment.
- Although the compound affinity to mutant kinases has been addressed frequently, compound residence time for mutants is rarely reported.
- Evaluating the residence time of kinase inhibitors at mutant as well as wild-type kinases in living cells is highly desired.

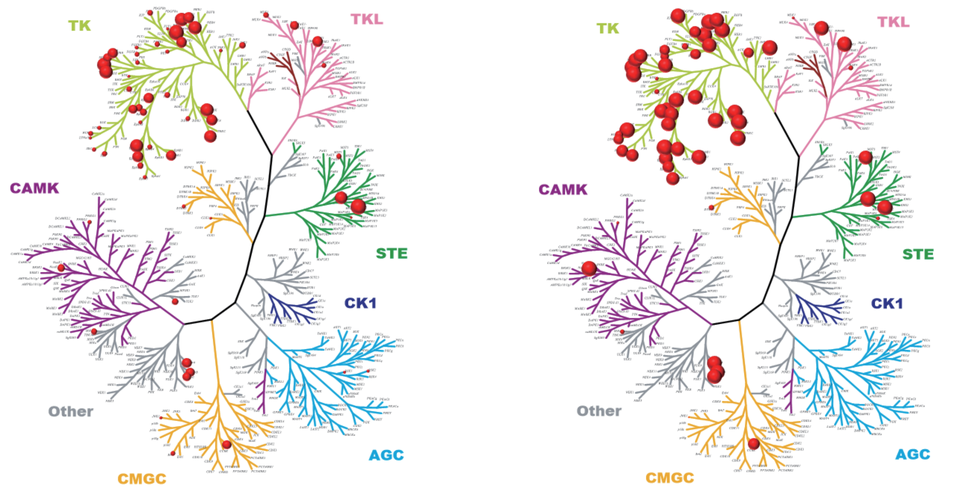
NanoBRET™ in Kinetic Mode



The NanoBRET™ TE Intracellular Kinase Assay utilizes BRET in living cells by molecular proximity of the NanoBRET™ Tracer to the NanoLuc® luciferase-fused kinase. After equilibrating with a near-saturating concentration of compound, the cells are washed to remove unbound compound and treated with a near-saturating concentration of a cell-permeable tracer. BRET is measured repeatedly in a luminometer to observe tracer association as a proxy for compound dissociation.

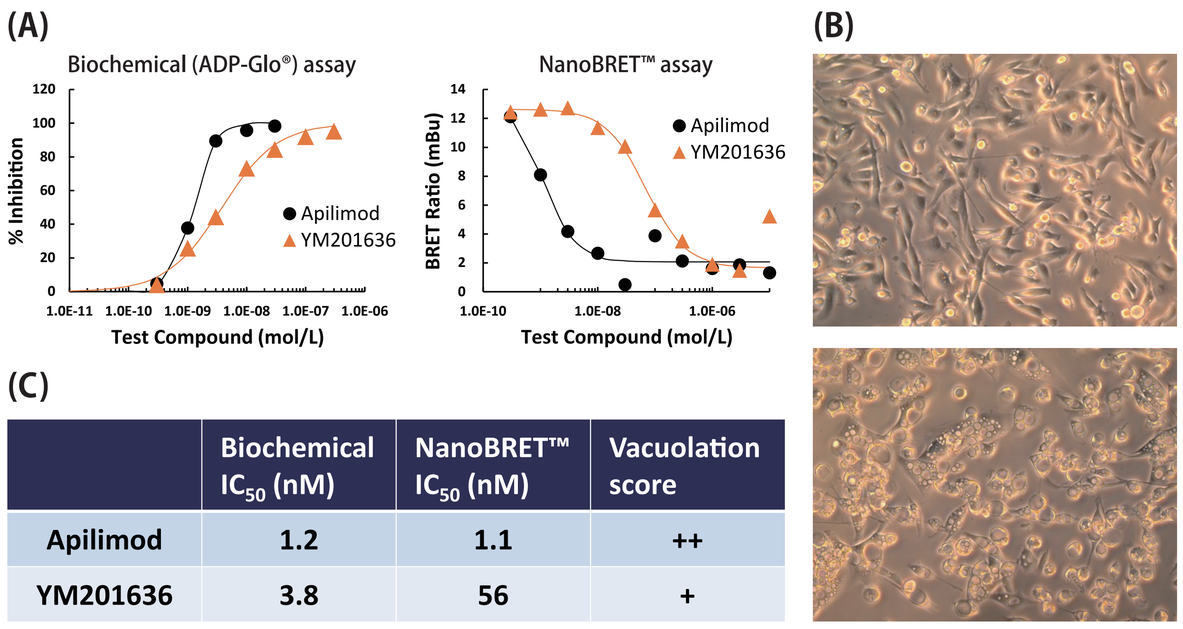
Result:

Figure 1. The NanoBRET™ assay reveals improved selectivity of crizotinib in living cells compared to biochemical assay.



A comparison of occupancy vs inhibition of 1 μM crizotinib in NanoBRET™ versus cell-free (QuickScout™, Carna Biosciences). In live cells, crizotinib is more selective for MET and ALK.

Figure 2. PIKFYVE inhibition induces vacuolation phenotype with a better correlation with the results in the cellular NanoBRET™ TE assay.

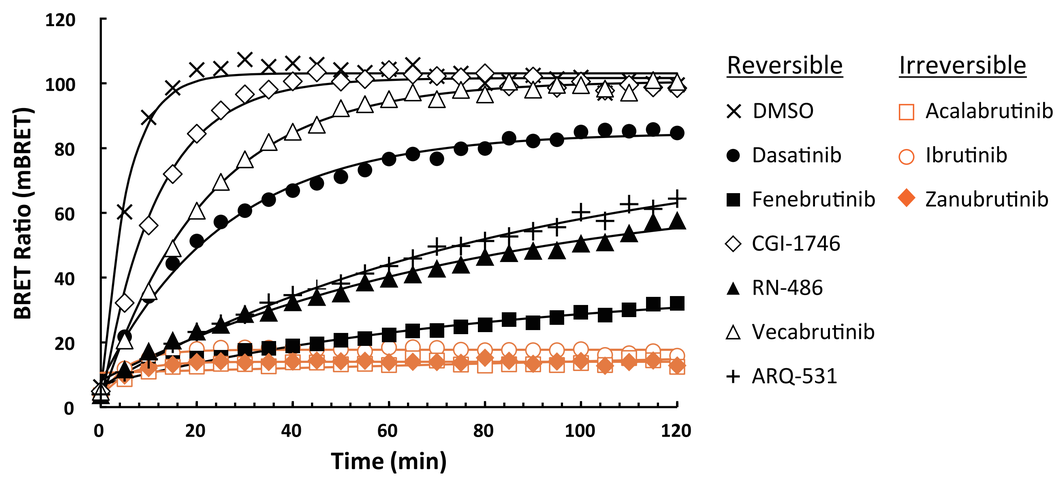


(C)

	Biochemical IC_{50} (nM)	NanoBRET™ IC_{50} (nM)	Vacuolation score
Apilimod	1.2	1.1	++
YM201636	3.8	56	+

(A) Biochemical enzyme (ADP-Glo®) and NanoBRET™ assay of PIKFYVE, a target now implicated in COVID-19 infectivity and viral entry. (B) PIKFYVE inhibition promoted cytoplasmic vacuolation. MDA-MB-231 cells were treated for 48 hours with DMSO (top) or 0.3 μM apilimod (bottom). (C) Vacuolation phenotype correlated well with the IC_{50} in the NanoBRET™ assay. Vacuolation phenotype was scored by inhibitor concentration at which almost all of cells in a field of view showed vacuolation when cells were treated for 48 hours with increasing dose of inhibitors (++: ≥ 0.3 μM, +: ≥ 3 μM).

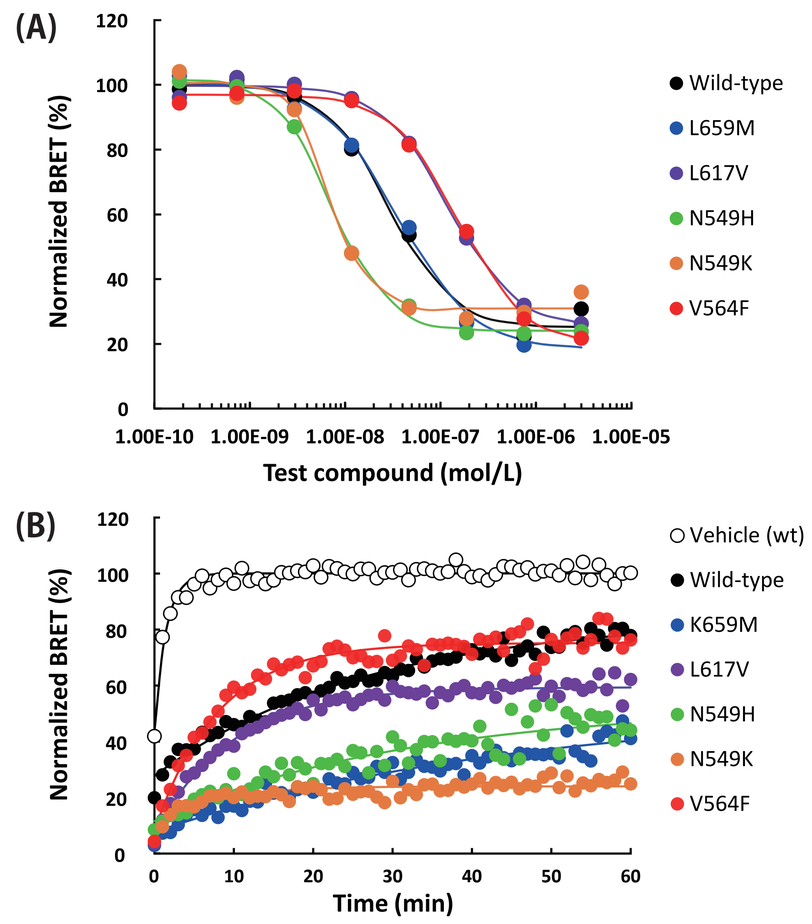
Figure 3. Drug-target residence time can be measured with reversible and irreversible inhibitors.



Compound	IC_{50} (nM)	Test concentration (nM)	Residence Time (min)
Dasatinib	20.0	100	68
Fenebrutinib	6.42	30	459
CGI-1746	66.6	1000	15
RN-486	6.46	30	179
Vecabrutinib	28.1	300	26
ARQ-531	33.4	300	134
Acalabrutinib	33.5	100	> 1,000
Ibrutinib	0.84	3	> 1,000
Zanubrutinib	1.93	10	> 1,000

Intracellular residence time analysis of reversible and irreversible BTK inhibitors. HEK293 cells expressing BTK were preincubated with test compounds or vehicle(DMSO) for 2 hours followed by a brief washout. The NanoBRET™ Tracer K-4 was then added and BRET was repeatedly measured with the Glomax® Discover Multimode Microplate Reader (Promega).

Figure 4. Residence time of ponatinib at FGFR2 remarkably varies between wild-type and mutants, and among the mutants.



Target	IC_{50} (nM)	Test concentration (nM)	Residence Time (min)
Wild-type	28.5	100	52
K659M	35.3	300	155
L617V	122	1000	32
N549H	7.26	30	144
N549K	7.09	30	>1000
V564F	156	1000	20

(A) Cellular target engagement profile of ponatinib against wild-type and various mutant FGFR2s interrogated using the tracer K-10. Data are normalized to the BRET ratio of the vehicle-treated sample. (B) Residence time analysis reveals remarkably slow dissociation of ponatinib from FGFR2 [N549K], compared to wild-type and other mutant FGFR2s. Data are normalized to the plateau value of the BRET recovery profile for the vehicle-treated sample in each assay.

Conclusions:

Carna Biosciences is offering target engagement intracellular kinase assay services using NanoBRET™ in collaboration with Promega. We can provide data of residence time as well as affinity of compounds against kinome-wide targets (> 200).

1. NanoBRET™ TE assays enable the quantitative determination of compound residence time in living cells.
2. Evaluation of drug-target residence time at both wild-type and mutant kinases is desirable to illustrate kinetic selectivity of test compounds, offering advantages in drug discovery programs.