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Kinase/Small Inhibitor interaction Evaluated Directly in Cell Lysates and in Whole Cells: A Combined Capillary Electrophoresis and Microscale Thermophoresis Study

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Summary

Cell life is orchestrated by molecular interactions that controls signaling pathways. Studying these interactions remains a challenge if one wishes to work under conditions close to in vivo. In this project, we have combined capillary electrophoresis (CE) and microscale thermophoresis (MST) to characterize protein/ligand interaction in terms of affinity and thermodynamics [1,2]. Our ambition was to succeed in these miniaturized assays by working directly with cellular lysate or even in whole cells, without radioactivity, immobilization steps, or purification steps.

For this purpose, we report and validate for the first time the use of the red fusion miRFP670 protein as a fluorophore for MST opening the way to original bioanalysis applications. Indeed, in contrast to the commonly used Green fluorescent protein (GFP) protein, miRFP670 allows to conduct MST analyses an optical window (650-900 nm) where background autofluorescence of cells is low [3]. We chose LIM kinases (LIMKs) as enzymes of interest because, despite their established implication in numerous pathologies [4], no inhibitor has passed the clinical trial stage, undoubtedly due to a lack of holistic understanding of these therapeutic targets. We successfully overexpressed miRFP670-LIMKs in HEK293 cells as confirmed by western blot analyses. Maintaining the catalytic activity of LIMK in the cellular lysate towards its substrate, cofilin, even after its tagging with miRFP670, was controlled using CE. A poly(diallyldimethylammonium) or PDADMAC-coating of the silica capillaries was mandatory for the developed CE method to be reliable. Cell lysates were then analyzed by MST, again without any purification step. K_d, ΔH, ΔS and ΔG values for LIMK interactions with various small inhibitors were successfully obtained. Very recently, we went further by conducting miRFP670-LIMK / inhibitor affinity assays directly within whole cells. This remarkable approach provides, in a straightforward manner, insights into the cellular permeability of a potential inhibitor.

Acknowledgement

The authors would like to thank QUALICHIM (APR-IA-PF 2021-00149467) and the Labex program SYNORG (ANR-11-LABX-0029) for their financial support of ICOA, UMR 7311, University of Orléans, CNRS.

References

[1] S. Duhr, D. Braun, Why molecules move along a temperature gradient, Proc. Natl. Acad. Sci. 103 (2006) 19678–19682.

[2] J.A.C. Stein, A. Ianeselli, D. Braun, Kinetic Microscale Thermophoresis for Simultaneous Measurement of

Binding Affinity and Kinetics, Angew. Chem. Int. Ed. 60 (2021) 13988–13995.
[3] D.M. Shcherbakova, V.V. Verkhusha, Near-infrared fluorescent proteins for multicolor in vivo imaging, Nat. Methods 10 (2013) 751–754.
[4] E. Villalonga, C. Mosrin, T. Normand, C. Girardin, A. Serrano, B. Žunar, M. Doudeau, F. Godin, H. Bénédetti, B. Vallée, LIM Kinases, LIMK1 and LIMK2, Are Crucial Node Actors of the Cell Fate: Molecular to Pathological Features, Cells 12 (2023) 805.