Presentation pathway of B2 metabolites by MR1 via Fluctuation Correlation Spectroscopy

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Mucosal-associated invariant T-cells (MAIT cells) are innate semi-invariant T-cells which comprise the 10% of the T-cell in peripheral blood. Despite the high population, their role in the disease response is not completely known. MAIT cells have a semi-invariant T-cell receptor (TCR) that can interact with metabolites of Vitamin B2 (Riboflavin). This behaviour is unexpected compared to the rest of the TCR which interact with macromolecules like peptides or glycolipids.[1,2]

The TCR need an antigen presenting molecule (APM) to be able to interact with this unstable metabolites. The major histocompatibility complex I-related (MR1) is the antigen presenting molecule which is responsible of stabilizing this non-endogenous metabolites and presenting them to MAIT cells.[3-5] MR1 pathway and metabolism is still not completely studied and it plays an important role in the MAIT cells activity[6,7].

In this work we explore the use of fluctuation correlation spectroscopy (FCS) to study the trafficking and interactions of the antigen (Vitamin B2 metabolites) with MR1[8-10]. FCS will allow monitoring the interaction of MR1 and VitB2 metabolites, specifically to identify the intracellular compartments where they associate and how they travel to the cell surface.

1 Le Bourhis, L. *et al.* Antimicrobial activity of mucosal-associated invariant T cells. *Nat Immunol* **11**, 701-708, doi:10.1038/ni.1890 (2010).
2 Leeansyah, E., Loh, L., Nixon, D. F. & Sandberg, J. K. Acquisition of innate-like microbial reactivity in mucosal tissues during human fetal MAIT-cell development. *Nat Commun* **5**, 3143, doi:10.1038/ncomms4143 (2014).
3 Eckle, S. B. *et al.* Recognition of Vitamin B Precursors and Byproducts by Mucosal Associated Invariant T Cells. *J Biol Chem* **290**, 30204-30211, doi:10.1074/jbc.R115.685990 (2015).
4 Franciszkiewicz, K. *et al.* MHC class I-related molecule, MR1, and mucosal-associated invariant T cells. *Immunological Reviews* **272**, 120-138, doi:10.1111/imr.12423 (2016).
5 Huang, S. *et al.* Evidence for MR1 antigen presentation to mucosal-associated invariant T cells. *J Biol Chem* **280**, 21183-21193, doi:10.1074/jbc.M501087200 (2005).
6 McWilliam, H. E. *et al.* The intracellular pathway for the presentation of vitamin B-related antigens by the antigen-presenting molecule MR1. *Nat Immunol* **17**, 531-537, doi:10.1038/ni.3416 (2016).
7 McWilliam, H. E., Birkinshaw, R. W., Villadangos, J. A., McCluskey, J. & Rossjohn, J. MR1 presentation of vitamin B-based metabolite ligands. *Curr Opin Immunol* **34**, 28-34, doi:10.1016/j.coi.2014.12.004 (2015).
8 Digman, M. A. & Gratton, E. Lessons in fluctuation correlation spectroscopy. *Annu Rev Phys Chem* **62**, 645-668, doi:10.1146/annurev-physchem-032210-103424 (2011).
9 Digman, M. A. & Gratton, E. Imaging barriers to diffusion by pair correlation functions. *Biophys J* **97**, 665-673, doi:10.1016/j.bpj.2009.04.048 (2009).
10 Hinde, E. & Cardarelli, F. Measuring the flow of molecules in cells. *Biophys Rev* **3**, 119, doi:10.1007/s12551-011-0051-x (2011).