

Quantitative FRET imaging of leptin receptor oligomerization kinetics in single cells

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Leptin is an adipocyte-secreted hormone that mediates its function through activation of its membrane receptor (LEPR) [1]. We present FRET results from the studies of leptin-induced events occurring in short (LEPRa) and long (LEPRb) isoforms of leptin receptors in the cell membrane. Leptin receptor isoforms, tagged at their C terminal with cyan (CFP) or yellow (YFP) fluorescent proteins were prepared. The resulting constructs, namely, mouse (m) LEPRa-YFP and -CFP, mLEPRb-YFP and -CFP were tested for biological activity in transiently transfected CHO and HEK 293T cells for activation of STAT3-mediated luciferase activity, phosphorylation of MAPK and binding of radiolabelled leptin. All four constructs were biologically active and were as potent as their untagged counterparts. The localization pattern of the fused protein appeared to be confined almost entirely to the cell membrane. The leptin-dependent interaction between various types of receptors in living cells were studied by measuring FRET, using fluorescence lifetime imaging microscopy (FLIM) and acceptor photobleaching (APB) methods.

Both methods yielded similar results indicating that: (i) leptin receptors expressed in the cell membrane exist mostly as preformed LEPRa/LEPRa or LEPRb/LEPRb homo-oligomer but not as LEPRb/LEPRa hetero-oligomers; (ii) the transient leptin-induced FRET appearance in cells transfected with LEPRb/LEPRb most likely reflects both conformational change that leads to closer interaction in the cytosolic part and higher FRET signal as well as *de novo* homo-oligomerization; (iii) in LEPRa/LEPRa exposure to leptin does not lead to any further increase in FRET signaling as the proximity of CFP and YFP fluorophores in space gives already maximal FRET efficiency of the preoligomerized receptors. An interesting aspect that makes our results novel is the time course studies of receptor homo-oligomerization kinetics. We observed in the case of LEPRb/LEPRb interactions, that the FRET signal increases to a maximum after 3-4 minutes of hormone addition and then decreases to a lower value. This transient FRET signal is reported for the first time in literature and this may have significant implications in the succeeding signaling events. Deeper insight into CFP lifetime data, using population analysis, further suggested that at least in the case of cells transfected with LEPRb-CFP/LEPRb-YFP both *de novo* oligomerization as well as conformational change of pre-oligomerized receptors occur simultaneously upon exposure to leptin [2].

References:

- [1] Zhang Y et al. *Nature* **372**, 425-432 (1994).
- [2] Biener et al. *Biology of the Cell*, (submitted) 2005.

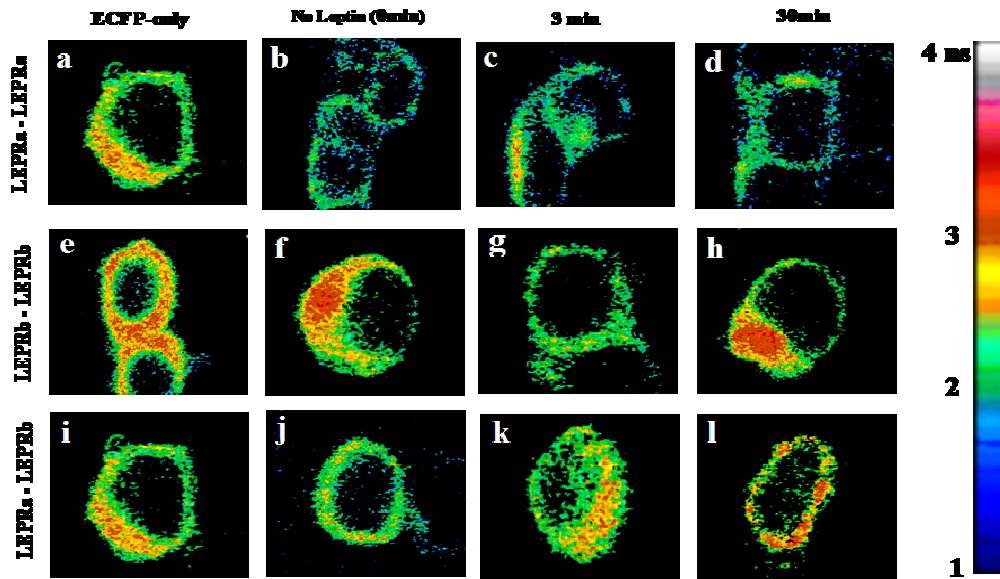


Fig 1: Representative fluorescence Lifetime (τ_t : t = leptin incubation time in minutes) images of HEK 293T cells coexpressing ECFP and EYFP tagged leptin receptor isoforms at three time points (0,3 and 30 minutes) after leptin stimulation. Cells expressing only ECFP are shown for reference lifetime (τ_o) images and energy transfer efficiency is calculated from the expression: $E = 1 - (\tau_t / \tau_o)$.

Figure 2A

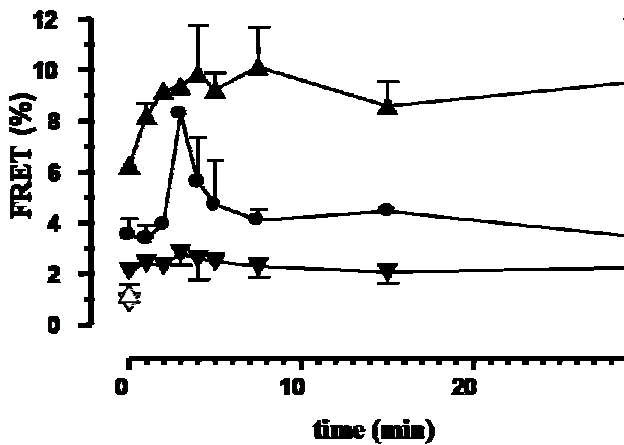


Fig 2A: Time course of FRET efficiency upon leptin stimulation as measured by acceptor photobleaching FRET in cells transfected with LEPRa-CFP and LEPRa-YFP (\blacktriangle), LEPRb-CFP and LEPRb-YFP (\bullet), LEPRa-CFP and LEPRb-YFP (\blacktriangledown) or with LEPRa-CFP (Δ) or LEPRb-CFP (∇) only. **Fig 2B :** Individual FRET efficiency values obtained from two FLIM experiments were pooled and then grouped into three populations: (A) No-FRET comprising of those regions whose mean value was FRET efficiency $\leq 4.16\%$ corresponding to instrument sensitivity limits (B) Low-FRET comprising of those regions whose mean value, $4.16\% \leq$ FRET efficiency $\leq 12\%$ and (C) High-FRET comprising of those regions whose mean value was $\geq 12\%$. Cells transfected with LEPRa-CFP and LEPRa-YFP (\square and full line) and cells transfected with LEPRb-CFP and LEPRb-YFP (\blacksquare and dashed line).

Figure 2B

