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In situ proximity ligation assay to study heteroreceptor complexes in the brain

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GProtein Coupled Receptors (GPCRs) and Receptor Tyrosine Kinases (RTK) play critical roles in cellular processes and signaling and have been shown to form homo and heteroreceptor complexes with diverge biochemical and/or pharmacological activities. However, despite of extensive experimental results supporting the formation of GPCR and/or RTK homo and heteroreceptor complexes in heterologous systems, the existence of such homo and heteroreceptor complexes in their native environment remains largely unknown, mostly because of the lack of appropriate methodology. For instance, until recent years the methods that have been developed to study receptor-receptor interactions require that genetic constructs be expressed in the cells to enable detection of the receptor interactions, thus excluding the use of tissue samples. In order to demonstrate in native tissue the existence of GPCR and/or RTK homo and heteroreceptor complexes, especially in a manner that can be generally applicable to different receptor pairs, a well-characterized in situ proximity ligation assay (in situ PLA) has been adapted to confirm the existence of GPCR and/or RTK homo and heteroreceptor complexes in brain slices ex vivo. We also describe the in situ PLA procedure as a high selectivity and sensitivity assay to image GPCR and/or RTK homo and heteroreceptor complexes in brain sections by confocal microscopy and how the assay is performed. We point out as well the method advantages and disadvantages and compare it to other available techniques.

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Quantitative systems biology approaches lead to efficient elucidation of transcriptional regulatory networks

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A computational genome-scale systems biology approach to experimental design was applied to elucidate the transcriptional regulation of nitrogen metabolism in Escherichia coli by two major transcription factors, NtrC and Nac. Two alternative nitrogen sources, cytosine and cytidine were predicted by genome-scale models to maximally activate the NtrC and Nac regulons and thus optimally elucidate their function. Genome-wide ChIP-exo and RNA-seq measurements were performed and 19, 249, 153 and 2171 binding sites for NtrC, Nac, RpoN and RpoD respectively were identified resulting in 262 new binding sites for NtrC and Nac., In addition to guiding experimental design, a genome-scale model of *E. coli* metabolism was used to gain a detailed quantitative understanding of how the entire metabolic network responds to different nitrogen sources in order to carry out its integrated function. While NtrC primarily responds to nitrogen limitation by striving to increase nitrogen availability, Nac rebalances metabolic fluxes through carbon metabolism to accommodate a nitrogen source change. The study shows how computational models based on system biology serve to both optimally design experiments to elucidate regulons and reveal the full physiological roles of transcription factors.

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