UNIVERSITEIT GENT

CHEMTECH LIFE SCIENCE

TECHNOLOGY OFFER

TRAPPING LIGAND-PROTEIN INTERACTIONS AT THE CELL-SURFACE

We are seeking partners interested in our furan crosslinking methodology, here specifically used for investigating interactions between ligands and cell-surface receptors, in view of potential applications in covalent membrane receptor targeting.

INTRODUCTION

Interactions between ligands, such as peptides or small molecules, and cell surface proteins, such as G-protein coupled receptors (GPCR), are crucial for numerous key processes in living organisms, making them attractive drug targets. The method-of-choice to observe the interaction between these receptors and their ligands is by the formation of a stable chemical bond between both binding partners (crosslinking). Traditionally used photocrosslinking moieties can give rise to a high background crosslinking and the obligatory use of harmful UV light can result in phototoxic effects when working in living cells.

Recently, a new crosslinking technology was developed based on the use of a furan moiety. Furan needs primary oxidative activation to allow covalent bond formation with nucleophilic sites in its proximity. The furan crosslinking was originally applied for oligonucleotide crosslinking but can also be applied in peptide-protein crosslinking (see figure 1)

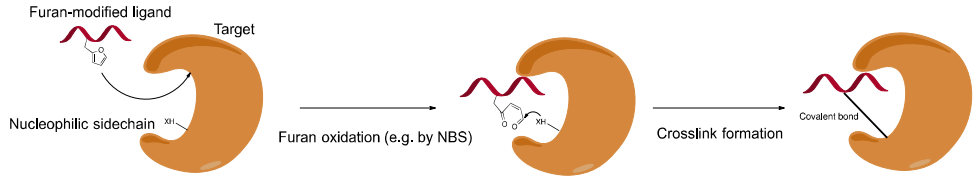

Figure 1: General principle of furan-crosslinking. Furan-modified ligands can be used for selective crosslinking with their respective binding partner. The inert furan moiety is activated by oxidation and reacts with nucleophilic groups to form a stable covalent bond between the two partner molecules.

TECHNOLOGY

To activate the unreactive furan moiety for enabling crosslink formation, an oxidation step is normally required. This oxidation can be performed using different methods, involving chemical oxidants or by the generation of singlet oxygen. With the present technology, one can use furan crosslinking to obtain a covalent bond between a ligand and its native cell-surface receptor without the need of such an external oxidation step. When working in living cells, the furan moiety, present in the ligand or in the cell-surface receptor, will be oxidized spontaneously upon formation of the ligand-receptor complex, and by this a highly specific crosslink will be formed between both binding partners.

APPLICATIONS

With the presented technology, several applications can be envisaged. At first, the possibilities for early-stage drug discovery are obvious. Freezing the interaction between a ligand and its cell-surface receptor by crosslinking, allows to influence the dynamic recognition process and should thus facilitate the study of the formed complex. The furan technology allows identification of new, possibly druggable, cell surface protein receptors of biologically active orphan ligands. Vice-versa, genetic incorporation of a furan moiety in a cell-surface receptor could make the identification of new drug candidates easier. Moreover, due to the site-selective nature of the crosslinking methodology, one could get further insight in the specific binding-site of a certain ligand.

Alternatively, this method could form a cheap, more reliable alternative to antibodies for visualization of cell-surface receptors. Due to dynamic trafficking of receptors, such as GPCRs, the glycosylated fractions present at the cell-surface are often difficult to visualize using a combination of primary and secondary antibodies. Using fluorescently labeled, furan-modified ligands, these under-represented fractions of the receptor could be visualized more easily and with a higher sensitivity.

ADVANTAGES

- ✓ Easy synthesis of furan modified (peptide) ligands, thanks to commercial availability and easy handling of building blocks.
- ✓ Site-specific crosslinking, hereby reducing background labeling and making it possible to precisely define binding site of ligand.
- ✓ Crosslinking in physiological conditions, which makes observation of biological interactions in 'native' conditions possible.
- ✓ Endogenous oxidation of the furan moiety, removing the need for extra manipulation of the cells.

STATUS OF DEVELOPMENT

The interaction between peptide ligand kisspeptin-10 and its native receptor, GPR54, was efficiently crosslinked using the furan oxidation technology.

A furan-modified kisspeptin peptide gave rise to a site-specific crosslink with the glycosylated subform of the receptor, present at the cell-surface.

In different mammalian cell lines, including cancerous as well as healthy cells, crosslinking was observed without the need of adding an external oxidizing signal, implying that oxidation of the furan moiety occurs by endogenous factors. Furan crosslinking was benchmarked against a classical photocrosslinking strategy, showing lower background and higher specificity.

PARTNERSSHIP

Further data are yet to be gathered concerning this invention and its possible applications. We invite industrial partners interested in this technology to collaborate and boost further discoveries in view of future products for the market.

INTELLECTUAL PROPERTY

The furan oxidation crosslinking methodology was earlier published as patent application and granted in the US (US9290537). An additional patent concerning the use of the technology for covalently binding a cell surface protein and a ligand is pending and to be published.

REFERENCES

Vannecke, W., Ampe, C., Van Troys, M., Madder, A. Crosslinking furan-modified kisspeptin-10 with the KISS- receptor: NOX does the trick, *in revision*

KEYWORDS

Crosslinking, GPCRs, cell-surface proteins, furan, orphan peptide

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