Biosensors and Bioelectronics 74 (2015) 757–763

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Micro-structured peptide surfaces for the detection of high-affinity peptide–receptor interactions in living cells

Anna-Maria Lipp [1], Bozhi Ji [1], Roland Hager, Sandra Haas, Simone Schweiggl, Alois Sonnleitner, Thomas Haselgrübler *

Center for Advanced Bioanalysis GmbH, Gruberstrasse 40, 4020 Linz, Austria

ARTICLE INFO

Article history:
Received 3 June 2015
Received in revised form
15 July 2015
Accepted 17 July 2015
Available online 18 July 2015

Keywords:
Photolithography
Live-cell assay
Micro-structured streptavidin
Micro-patterning
Cell adhesion
Peptide–receptor interaction

ABSTRACT

Peptide ligands have great potential as selective agents for diagnostic imaging and therapeutic targeting of human cancers. A number of high-throughput assays for screening potential candidate peptides have been developed. Although these screening assays are indispensable for the identification of peptide ligands at a large scale, it is crucial to validate peptide binding and selectivity for targeted receptors in a live-cell context. For testing high-affinity peptide-receptor interactions in the plasma membrane of living cells, we developed cell-resistant, micro-structured glass surfaces with high-density and high-contrast peptide features. Cell adhesion and recruitment of fluorescent receptors to micro-patterned peptides in the live-cell membrane were evaluated by reflection interference contrast (RIC) and total internal reflection (TIRF) microscopy, respectively. To demonstrate both the specificity and modularity of the assay, co-patterning of fluorescent receptors with three different immobilized micro-structured ligands was shown: first, interaction of green fluorescent protein (GFP)-tagged epidermal growth factor (EGF) receptor expressed in Jurkat cells with immobilized EGF was detected and quantified. Second, using Jurkat cells, we demonstrated specific interaction of yellow fluorescent protein (YFP)-tagged β3 integrin with c (RGDfK) peptide. Third, we identified indirect recruitment of GFP-tagged α5 integrin to an 11-mer peptide. In summary, our results show that the developed micro-structured surfaces are a useful tool for the validation and quantification of peptide–receptor interactions in their natural cellular environment.

1. Introduction

Cell surface receptors are important selective targets for around 60% of all drugs in clinical use (Overington et al., 2006). Thus, identifying ligand–receptor interactions constitutes an extremely active area of research. Since a variety of receptors are specifically overexpressed on the tumor cell surface (Reubi, 2003), natural and synthetic receptor-targeting peptides hold great potential for *in vivo* targeting of human cancers.

To date, a number of peptide candidates have been identified. Classically, peptide ligands for a specific target receptor are identified by high-throughput peptide screening tools like phage display or the yeast two-hybrid system. These library-based assays use multiple rounds of selection involving removal of unbound and amplification of bound candidates to enrich for high-affinity peptide ligands (Szardenings, 2003; Takahashi and Roberts, 2009). However, the isolation of whole membrane receptors as targets in the *in vitro* settings of library screening is very challenging (Molek et al., 2011). Moreover, the membrane integrity of proteins is crucial for their binding characteristics (Lee, 2004; Savas et al., 2011). Although panning against whole cells can be used to probe for peptide binding to the target receptor in its native conformation (Szardenings et al., 1997), this technique requires relatively large numbers of cells, and ligands may be lost due to repeated washing steps (Clackson and Lowman, 2004). Therefore, focused experiments using small cell numbers without the need for washing would be a beneficial complement to large-scale methods for validating candidate interactions in a live-cell context. Thus, we developed an easy-to-implement and scalable assay to directly test for specific peptide–receptor interactions in the membrane of living cells.

Capturing membrane receptors by micro-patterned proteins has been shown to be a suitable tool for the detection and quantification of protein–protein interactions in live-cell membranes (Lanzerstorfer et al., 2014; Löchte et al., 2014; Schwarzenbacher et al., 2008; Singhai et al., 2014). So far, micro-structured peptide surfaces have been described for the spatially resolved adhesion

* Corresponding author. Fax: +43 732 2468 7530.
E-mail addresses: anna-maria.lipp@cbl.at (A.-M. Lipp), bozhi.ji@cbl.at (B. Ji), roland.hager@cbl.at (R. Hager), sandra.haas@cbl.at (S. Haas), simone.schweiggl@cbl.at (S. Schweiggl), alois.sonnleitner@cbl.at (A. Sonnleitner), thomas.haselgruebler@cbl.at (T. Haselgrübler).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.bios.2015.07.038
0956-5663/

and growth of cells, as well as for the improvement of vascular biomaterials. Micro-contact printing (Csucs et al., 2003), inkjet-printing (Boivin et al., 2013) and spraying (Hoesli et al., 2014) have been used as micro-structuring methods. Here we employed photolithography for micro-structuring peptides on glass surfaces and used these surfaces for the validation of high-affinity peptide–receptor interactions in living cells. Using TIRF-based live-cell imaging, specific peptide–receptor interactions were detected by measuring the spatial redistribution of the fluorescently tagged receptor in the cell membrane according to the micro-patterned peptides linked to the glass surface and analyzed quantitatively *via* co-localization analysis. This approach has great potential to complement traditional peptide screening methods for the validation and quantification of strong peptide binders in the physiological context of a living cell.

2. Materials and methods

The chemicals and reagents used are listed in Supplementary information.

2.1. Preparation of micro-structured peptide surfaces and determination of peptide density plus pattern contrast

Micro-structured peptide surfaces were prepared as outlined in Fig. 2. In short, PEG was grafted onto epoxy-functionalized glass coverslips, blocked with ethanolamine and followed by biotinylation of terminal amine groups on the PEG brush. Biotin microstructures were generated by photolithography using positive photoresist. PEG was re-grafted in the developed areas. Biotinylated peptides were bound to the micro-structured surface *via* biotin–streptavidin (STA) interaction. Peptide density and pattern contrast were determined both at the STA and peptide level using fluorescence microscopy. Surface preparation and quality control is detailed in Supplementary information.

2.2. Plasmids, cell culture, and transfection

Jurkat cell lines were maintained in complete RPMI medium. Cells were transfected with plasmid DNA by nucleofection. GFP- and YFP-positive cells were enriched by flow cytometry and positive cells were selected using G418. Detailed procedures and information about used plasmids are listed in Supplementary information.

2.3. Assay readout and analysis

Live-cell experiments were performed on a custom-modified Zeiss Axiovert 200 microscope. Optical components and experimental setups for RIC and TIRF microscopy are described in detail in Supplementary information. Pearson cross-correlation analysis was performed to quantify co-localization between peptide (Cy5) and receptor areas (EGFP/EYFP) on a cell-by-cell basis as detailed in Supplementary information. Statistical analyses were performed using SigmaPlot (Systat Software Inc., San Jose, USA).

3. Results and discussion

3.1. Requirements for micro-structured peptide surfaces and quality control

Antibody micro-structured surfaces have been successfully used for the detection of protein–protein interactions in living cells by us (Lipp et al., 2014; Schwarzenbacher et al., 2008) and others (Lanzerstorfer et al., 2014; Sunzenauer et al., 2013). Here we developed micro-structured peptide surfaces for the detection of

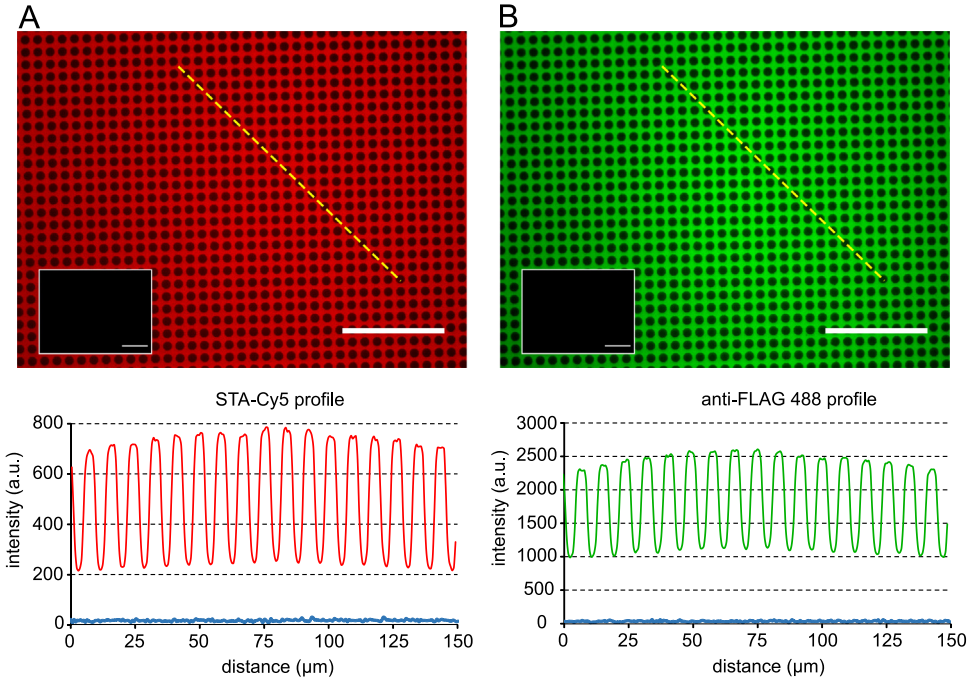

Fig. 1. Determination of biomolecule density and pattern contrast on micro-structured slides *via* fluorescence microscopy. (A) Density and contrast of STA was assessed using Cy5 labeling. Representative image of a micro-structured slide showing highly specific binding of STA–Cy5 in the grid area resulting in a high signal and pattern contrast (see line profile below). The inserted image represents the auto-fluorescence of the micro-structured surface in the Cy5 channel before coupling of STA–Cy5 (blue line). (B) Density and contrast of immobilized FLAG peptide was assessed using anti-FLAG AF488 on the same slide. Highly specific binding of FLAG peptide resulted in a high signal and pattern contrast of anti-FLAG AF488. The inserted image shows the signal of unspecific anti-FLAG AF488 binding without FLAG peptide (blue line). The dashed yellow line marks the position where the line profile was drawn. Scale bar = 50 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)