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Surfaceome nanoscale organization and extracellular interaction networks

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The reductionist view of 'one target-one drug' has fueled the development of therapeutic agents to treat human disease. However, many compounds that have efficacy *in vitro* are inactive in complex *in vivo* systems. It has become clear that a molecular understanding of signaling networks is needed to address disease phenotypes in the human body. Protein signaling networks function at the molecular level through information transfer via protein-protein interactions. Cell surface exposed proteins, termed the surfaceome, are the gatekeepers between the intra- and extracellular signaling networks, translating extracellular cues into intracellular responses and vice versa. As 66% of drugs in the DrugBank target the surfaceome, these proteins are a key source for potential diagnostic and therapeutic agents. In this review article, we will discuss current knowledge about the spatial organization and molecular interactions of the surfaceome and provide a perspective on the technologies available for studying the extracellular surfaceome interaction network.

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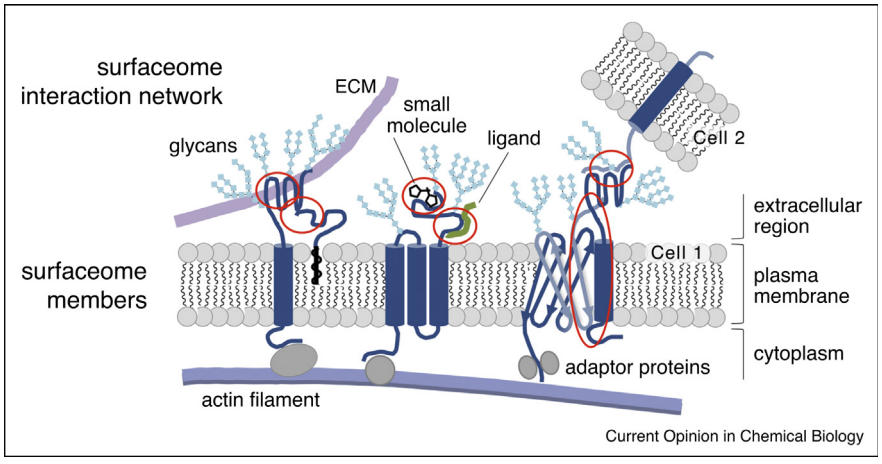
The extracellular surfaceome signaling interaction network

The surfaceome represents a signaling gateway for the cell that enables, but also limits, cellular communication to guide cellular behavior. Identity, quantity, and the spatiotemporal nanoscale organization of the surfaceome members determine cellular identity and the capacity of individual cells to communicate with the outside microenvironment. Although the amino acid sequence of surfaceome-residing proteins determines primary structure, the signaling function capacity is embedded in the dynamic interactions of surfaceome proteoforms with intracellular, lateral, and extracellular ligands. The elucidation of context-dependent signaling and dynamic molecular interactions of and within the surfaceome would provide the basis for targeted pharmacological interventions. In this review, we will focus on the extracellular signaling interaction network encompassing cis (or lateral) and trans (or extracellular) interactions. We acknowledge that this review paritally neglects the vast knowledge accumulated in the community about intracellular interactions of the surfaceome.

The human surfaceome encompasses all plasma membrane proteins with at least an extracellularly exposed amino acid. These extracellular residues decorated with a few or many post-translational modifications, have the general capacity to interact with proteins or other ligands from other cells (in trans), with the extracellular matrix (ECM), drugs, hormones and chemical messengers, as well as with lateral interaction partners (in cis) on the same cell (Figure 1). This cell-specific extracellular signaling interaction network regulates spatiotemporal signaling capacity and is influenced by the local microenvironment, including the lipid composition of the membrane, which enhances or limits fluidity [1,2], by the intracellular cytoskeleton, which restricts the localization of surfaceome proteins to confined spaces [3], and the ECM, which bridges surfaceome proteins [4•], as discussed below.

To define the protein members of the human surfaceome and thus the framework for protein-protein surfaceome interactions, we recently applied a machine-learning strategy and predicted that about 2900 proteins of a total of approximately 20,000 UniProt annotated protein entries belong to the surfaceome across all cell types and developmental stages (Bausch-Fluck *et al.*, The *in silico* Human Surfaceome, under revision; wlab.ethz.ch/surfaceome). Of these, 1500 were experimentally confirmed in a cell type-specific screen, in which individual cell types expressed between 500 and 1700 cell surface proteins [5]. The signaling interaction networks of individual receptors, such as the T cell receptor (TCR) as a prime example, have been studied extensively. Studies have revealed a remarkable complexity in terms of surfaceome interactions (involving at least 10 different surfaceome members in the case of TCR) that control signal initiation and regulation [6,7]. G protein-coupled receptors (GPCRs) engage in diverse surfaceome interactions with receptor activity-modifying

Figure 1



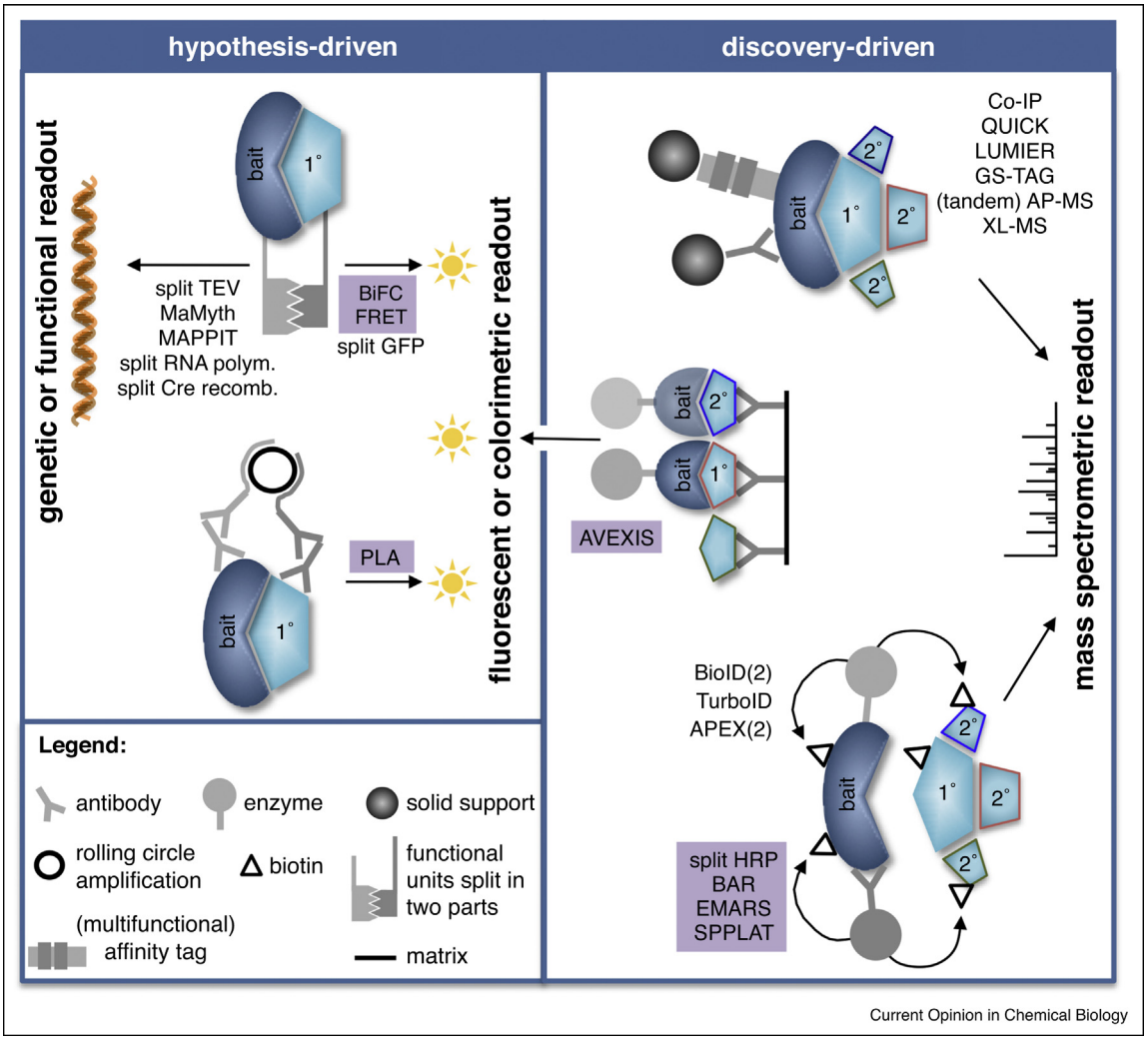
Surfaceome signaling interaction network. The surfaceome signaling network involves interactions of surfaceome proteins with the ECM, small molecules, ligands, cell surface proteins from other cells (trans interactions), and cell surface proteins from the same cell (cis interactions). Interaction sites are highlighted with red circles. Cell surface proteins are shown in dark blue, α-helical transmembrane domains are depicted by cylinders, β-barrel transmembrane domains by lines.

proteins (RAMPs), which guide localization of GPCRs and modulate signaling [8•]. Tetraspanins, cell surface proteins with four transmembrane domains, have the ability to interact with each other and with many signaling and adhesion surfaceome proteins, essentially spinning a web over the cell surface [9,10]. Hepatitis C virus (HCV) and human immunodeficiency virus (HIV) engage several cell surface proteins (CD81, SR-B1, CLDN1, Occludin and EGFR in the case of HCV [11] and CD4, CCR5, and CXCR4 in the case of HIV-1 [12,13]) to infect cells. Based on these examples, it appears likely that also less intensively-studied receptors engage in a multitude of lateral surfaceome interactions, which, however, are largely unknown. Single receptors can provide signaling activity, but nanoclusters ensure specificity of signaling [14••] by distinguishing background noise from real input signals and by integrating multiple cues from the extracellular or from the intracellular space of the cells. In addition, the surfaceome is highly dynamic, as observed during the TCR activation process. TCR activation is dependent on clustering of several molecules and the first phosphorylation events are detected just 5 s after stimulation, and co-activation and re-organization of TCR signaling proteins is observed after 30 s [15]. Thus, surfaceome interactions are highly dynamic and enable fine-tuning of cellular signaling through organizational changes. It is essential to elucidate the nanoscale organization of the surfaceome signaling interaction network to understand how cells regulate signaling within specific spatial contexts; however, the efforts to obtain a systematic view of the surfaceome signaling interaction network and its spatiotemporal dynamics are still in their infancy.

Technologies for the interrogation of the surfaceome interaction network

The direct functional consequences of dynamic protein–protein interactions sustains interest in the field of interactomics, and a plethora of technologies for the detection of direct and proximal interactions have been recently developed. Particularly, advancements in mass spectrometry (MS) based interactomics technologies have paved the way for large-scale interaction network studies [16–18]. Methods to study protein-protein interactions can be divided into hypothesis-driven technologies, in which interacting proteins (usually two) are engineered to enable detection, and discovery-driven technologies, in which one protein is engineered or endogenously targeted and a multitude of interactors are identified (Figure 2). Box 1 provides a general overview of technologies used to identify protein-protein interactions, and a list is provided in Table S1 in supplementary material. A more comprehensive review can be found in Eyckerman *et al.* [19]. Most current approaches were designed to elucidate the intracellular interaction network. Technologies specially designed to probe the extracellular ligand space in a discovery-driven manner are AVEXIS and the ligand-receptor capture (LRC) technologies [20–22]. Affinity-purification coupled to mass spectrometry (AP-MS) methods, usually employed in the context of intracellular interactions, can in principle be also applied to cell surface proteins, by affinity tagging the cytoplasmic domains [23,24,25,26•]. However, AP-MS methods typically utilize detergents for disrupting the membrane and isolating surfaceome complexes, a step that may disrupt transient or lipid-mediated interactions of surfaceome complexes.

Figure 2



Technologies for the identification and validation of protein-protein interactions. Illustration summarizing different concepts of binary and multiple methods. For each conceptual approach some representative methods are listed. The complete list can be found in Table S1 in supplementary material. Methods suitable for surfaceome signaling interaction network interrogation are highlighted in purple.

Proximity-based tagging technologies, which can be used to tag proximal proteins, for example through biotinylation, in their natural environment, are advantageous for the elucidation of the surfaceome signaling interaction network, since these technologies do not require the protein complex to remain intact for subsequent analysis. Proximity-based strategies such as BirA ligase-based and APEX/APEX2 technologies are currently being used mainly for the tagging of intracellular domains of cell surface proteins and thus would be only able to biotinylate intracellular proteins and neighboring cell surface proteins that have proximal cytoplasmic domains [27,28]. EMARS [29], split HRP [30], SPPLAT [31], and BAR [32] strategies have been applied for the tagging of extracellular domains and discovery of lateral surfaceome interactions. These proximity-tagging technologies in combination with an MS read-out strategy can provide a basic blueprint of the surfaceome interaction network. Proposed interactions could be further validated by hypothesis-driven methods such as a fluorescent read-out strategy like the proximity ligation assay (PLA) [33], split eGFP [34], BiFC [35], or FRET [36]. In summary, a number of technologies hold the promise for elucidating and validating the surfaceome signaling interaction network; however, specific challenges remain, as discussed in the next section.

Modulators of the surfaceome signaling interaction network

The surfaceome protein abundance

The first step in the definition of the surfaceome signaling interaction network is typically the identification of

Box 1 Overview of technologies for identification of surfaceome networks.

In most hypothesis-driven methods the bait and the prey are both genetically engineered to be complementary parts of a functional unit. Upon interaction of bait and prey, the two tagged parts may unite to produce a fluorescent signal (i.e. split GFP [34], FRET [36], BiFC [35], and BRET [37]) or to activate directly or indirectly the transcription of a reporter gene (i.e. MaMTH [38], split RNA polymerase [39], and split TEV [40]). The reporter gene can ensure survival or can produce a fluorescent, luminescent (split luciferase) [41], or colorimetric (split lactamase) [42] signal. Details about optogenetic methods can be found in the recently published reviews by Wiens and Campbell [43] and Snider *et al.* [44] (Figure 2, Table S1 in supplementary material). The proximity ligation assay (PLA) [33] relies on antibodies that recognize two proteins that may interact. The antibodies are tagged with short single-stranded DNA oligonucleotides, which are ligated and serve as a template for PCR amplification. The template is detected with fluorescent PLA probes when gathered in proximity. Inherent to all hypothesis-driven technologies is that there is a distance constraint within which interactions can be identified. For most of these technologies, a direct interaction of bait and prey protein is necessary. For PLA, the distance must be closer than 40 nm. In FRET, the donor and acceptor have even to be closer than 10 nm and must face each other in the right conformation in order for resonance energy transfer to occur (Table S1 in supplementary material).

With the rise of mass spectrometry-based proteomics, affinity-based methods have gained considerable momentum [45]. Typically, the bait protein is engineered with a (multifunctional) affinity tag (i.e. c-myc, FLAG, HA, strep-tag) that allows purification under mild lysis conditions preserving the interaction with prey proteins [45]. Purified prey proteins are digested into peptides, and identified by mass spectrometry. Variants of this approach include use of the combination of several affinity tags for tandem purification (TAP) or the purification of endogenous proteins with respective antibodies coupled to a solid support (QUICK, coIP) [46]. AP-MS has been proven to be extremely powerful in identifying protein networks based on stable interactions [16–18].

Recently, proximity-tagging approaches have become popular as these methods can detect transient protein-protein interactions. Proximity-based methods either rely on a promiscuous biotin ligase, which is genetically fused with a target protein (i.e. BioID [27], TurboID [47], split BioID [48], PUP-IT), or engage a peroxidase, which metabolizes a biotinylated substrate for radical-based biotinylation of proximal proteins (APEX2 [28], EMARS [29], SPPLAT [31], BAR [32]). Biotinylation by either approach allows for streptavidin-based protein purification, digestion, and subsequent identification by mass spectrometry. Biotinylation of proteins is achieved within a radius of approximately 40 nm of the bait, and, therefore, the detected interactions are not necessarily direct.

A last category contains methods specifically designed to identify extracellular interactions. In AVEXIS, the extracellular domains of cell surface proteins are engineered in two flavors, bait and prey, in order to probe all protein domains against each other [20]. Ligand-receptor capture methods such as TRICEPS-LRC and HATRIC-LRC employ a trifunctional linker enabling attachment of a ligand, covalent binding to the receptor, and solid-phase enrichment [21,22].

All described technologies and their characteristics are listed in Table S1 in supplementary material.

surfaceome members and thus possible network participants. The Cell Surface Protein Atlas (CSPA, http://wlab.ethz.ch/cspa/) [5] encompass the experimentally validated surfaceome. A comparative analysis of the cellular surfaceomes contained in the CSPA revealed firstly that surfaceomes of evaluated cell types are qualitatively highly similar and secondly that differences between cell types are mainly found in quantities of surfaceome proteins [5]. This suggests that the same proteins within the surfaceomes of different cells experience different protein neighborhoods. In other words, because of quantitative differences, the number of possible functional surfaceome interactions will vary from cell type to cell type, ultimately leading to different signaling thresholds and functional signaling capacities. Examples supporting this hypothesis include interactions of the ERBB2 (also known as HER2) receptor with the ERBB3 (also known as HER3) receptor [26•] and with integrin beta 1 [49••] in an abundance-dependent fashion. Modulation of abundances of cell surface proteins likely regulates complex formation and signaling capacities. Surfaceome nanoclusters might be composed of stable core interactors and dynamic interactors that change depending on cell identity [50•]. Therefore, surfaceome signaling interaction networks, receptor nanoclusters, and receptor activation mechanisms should be regarded as cell-type specific, which complicates the quest to elucidate the spatiotemporal surfaceome signaling interaction network.

The surfaceome structure

Superfast single-particle tracking (SPT) has revealed cytoskeleton-mediated compartmentalization of the plasma membrane [51•]. The so-called fences and pickets model proposes that a mesh controlled by actin creates microdomains of 30–200 nm between which proteins move via hop diffusion [51•]. Recently, CD44 was identified as a picket protein [4•]. A different, but not contradictory, model proposes that compartmentalization is mediated by lipid-sequestered 10 nm–200 nm sized nanodomains. These lipid rafts are enriched in cholesterol and sphingolipids and harbor receptors at higher density than non-lipid-sequestered regions and facilitate oligomerization and thus activation of signal transduction [52].

The organization of the plasma membrane creates different microenvironments that directly influence the activation and the signaling of receptor tyrosine kinases as reviewed by Casaletto and McClatchey [53•]. Simultaneous single-molecule tracking of G-protein-coupled receptors and G-proteins has revealed firstly that transient interactions occur in various confined spaces in the plasma membrane, secondly that agonists increase the association rate, and thirdly that signaling is only initiated in hot spots [54••]. The possibility that interactions with various membrane components regulate receptor activation imposes another level of complexity on control of receptor activation [55]. Interactions might not only change with cell identity, but receptors, and specifically receptor proteoforms, may also display spatial selectivity on the surface of the same cell constrained by different membrane compartments. Thus, available evidence

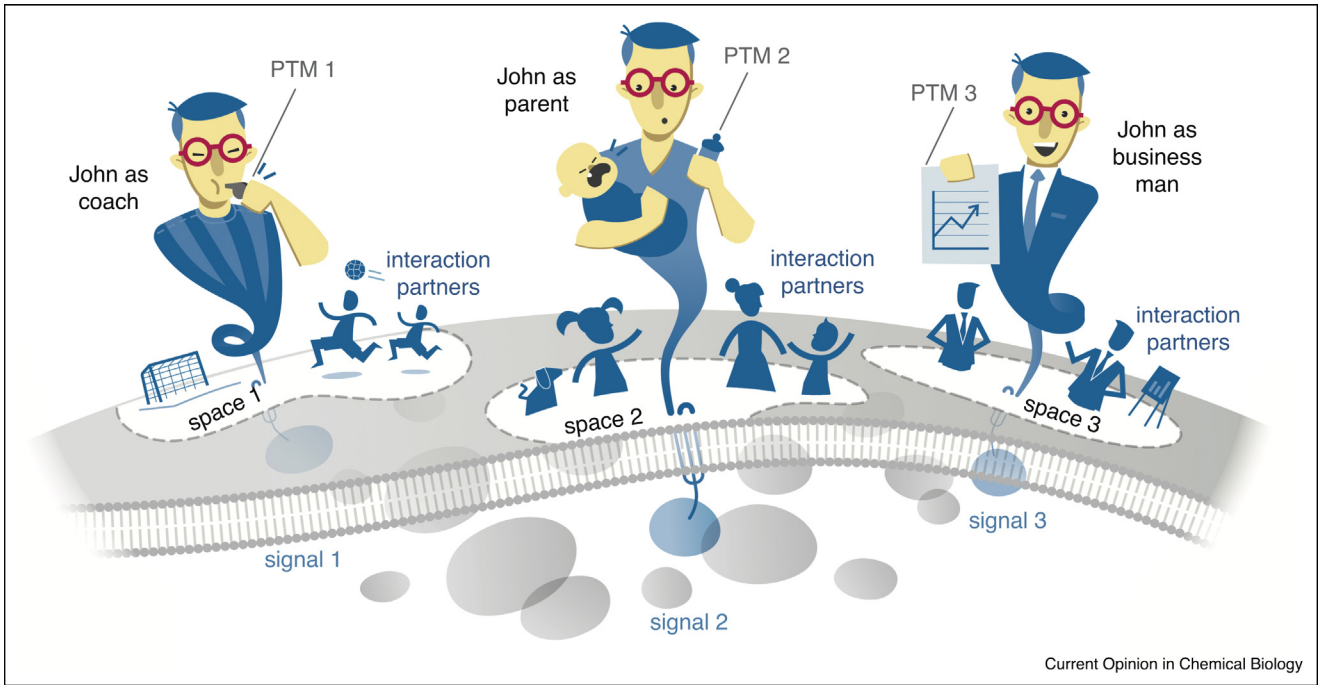
suggests that receptor proteoforms reside in spatially restricted surfaceome areas that contain, depending on the cell type, varying quantities of interaction partners; this compartmentalization ultimately leads to functionally different signaling outcomes in spatially separated nanoscale regions (Figure 3).

A consequence of such a scenario is that experimental strategies need to be devised and technologies developed that have the capacity to target selected, functionally relevant proteoforms of a receptor, rather than its total pool. Such data would enable the design of pharmacological interventions targeting specific perturbed signaling functions of a receptor and not the potentially positive signaling functions in non-perturbed nanoscale regions of the same cell.

Post-translational modifications of surfaceome proteoforms

To introduce a further level of complexity to the picture, single genes do not encode single protein forms. The term proteoform was coined to describe the related protein molecules arising from a single gene: The protein products from a single gene differ due to genetic variation, alternative processing of pre-mRNA, and post-translational modifications (PTMs) [56]. The main PTMs found on surfaceome-residing proteoforms are glycosylation (N-glycosylations, O-glycosylations, and C-glycosylations), disulfide bonds, lipidation, and phosphorylation with enrichment factors over intracellular proteins of 7.5, 6, 3, and 0.71, respectively (Bausch-Fluck *et al.*, unpublished, calculated based on average frequency within 100 amino acids). Glycans are involved in fundamental functions of the surfaceome such as cell signaling and communication, cell-matrix interactions, and immune modulation [57]. Alterations in glycosylation regulate the development and progression of cancer, and therapeutics have been developed that target the glycosylation pathway, gangliosides, or proteoglycans [58]. As examples of the importance of PTMs, EGFR glycosylation influences the conformation of the extracellular domain [59•] and ligand binding [60], and the conformation and thus the stability of interactions of integrins is influenced by N-glycosylation [61]. Since the majority of surfaceome proteoforms are glycosylated (90%), it is assumed that glycosylation has a substantial impact on the surfaceome signaling interaction network. For instance, glycan-binding galectins are capable of forming lattices over the cell surface [62] and have been proposed to scaffold cell surface domains to modulate signaling of the TCR,

Figure 3



Adaptation to different functional roles dependent on microenvironments and PTMs. The same protein (here symbolizes as the person John) can appear in different confined areas, where it possibly interacts with the available proteins (football players, family, business partners) within the same nanospace (space 1, space 2, space 3). Interactions with different proteins might be enabled by varying PTMs (whistle, bottle, documents). The changing interaction partners lead to modulated signals (signal 1, signal 2, signal 3). This figure summarizes how the structure of the surfaceome and PTMs in nanoscale regions influence the signaling capacity of individual proteoforms. The influence of the abundance of a protein on its interaction partners is not visualized. Image courtesy by *Tobias Fluck Graphic & Motion Design*.

among other pathways [63]. In order to deduce a complete surfaceome signaling interaction network and to understand its function, the proteoforms involved and their influence on signaling need to be integrated into the model.

The surfaceome signaling interaction network: what's next?

Large-scale interaction studies revealed on average 8–10 interaction partners per protein [16–18]. Except for some extensively studied receptors like EGFR, which engages in direct interactions with at least seven other surfaceome members [64•], the nanoscale organization and interaction partners of surfaceome proteoforms are largely unknown. As discussed, the surfaceome signaling interaction network is likely cell type, protein abundance, microenvironment, and proteoform-specific. In discovery-driven technologies and experimental design the obtained data are typically averaged across all the receptors, regardless of the microenvironment or the actual proteoform of the specific receptor. Technologies that allow targeting of proteoform-specific interaction pairs do not allow for the discovery of additional interaction partners. However, the influence of PTMs and the promiscuity of surfaceome proteins must be considered in order to deduce functionally relevant surfaceome interaction networks. Therefore, novel, advanced approaches are needed in the field. Along with new technologies, appropriate model systems need to be developed as well. For practical reasons, most of today's interaction studies are carried out in 2D cultures of cancer cell lines, which fall short in mimicking the natural microenvironment. However, it seems obvious that the surfaceome nanoscale organisation is adapted to interact with the surrounding microenvironment.

A recent single-molecule technique to probe protein-protein interactions appears to be promising [65]. Biotinylated antibodies against endogenously expressed proteins are used to immobilize complexes associated with the target proteins on a neutravidin-coated coverslip. Interacting proteins are detected by additional antibodies and total internal reflection fluorescence (TIRF) on each single spot. In addition, fluorescently labeled preys can be added to the array if no antibodies are available. The achieved single-spot resolution is able to dissect the different complexes involving a receptor of interest. However, only stable and anticipated interactions are able to be captured. Ideally, proximity technologies that are applicable in the extracellular space should be tailored to specific heterodimers or protein complexes, for example with engineered multispecific antibodies [66]. Antibody-based anti-cancer therapies are suffering from low perfusion rates of tumors, which results in low effectiveness in solid tumors, in high interstitial fluid pressure, and in the rapid development of drug resistance. A profound understanding of the spatiotemporal regulation of the surfaceome signaling interaction network and the synergistic and antagonistic effects of lateral surfaceome interactions would allow for the development of advanced drugs [66]. Multitargeted drugs should be less likely to induce resistance and should be more effective than drugs that target only a single proteoform. Multispecific antibodies can additionally be coupled with cancer cell-specific drugs for effective tumor clearance [67,68,69•].

Conclusions

The surfaceome signaling interaction network is tightly regulated, highly interactive, and immensely complex. As stated by de la Serna *et al.* [70]: 'A dynamic and complex plasma membrane is the environment where all molecules play in concert to achieve the optimal physiological output.' In order to understand and dissect this concert into its individual orchestral parts and to identify those parts that can be targeted to interfere with disease progression, advanced technologies are required that take into account the various particularities of the surfaceome. A better understanding of the surfaceome signaling interaction network will allow for the development of drugs that target functionally relevant surfaceome complexes.

Conflict of interest statement

Nothing declared.

Unicited reference

[71]

Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi:https://doi.org/10.1016/j.cbpa.2018.09.020.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Cooper RA: **Influence of increased membrane cholesterol on membrane fluidity and cell function in human red blood cells**. *J Supramol Struct* 1978, **8**:413-430.

2. Maity PC, Yang J, Klaesener K, Reth M: **The nanoscale organization of the B lymphocyte membrane**. *Biochim Biophys Acta* 2015, **1853**:830-840.

3. Duncan AL, Reddy T, Koldsø H, Hélie J, Fowler PW, Chavent M, Sansom MSP: **Protein crowding and lipid complexity influence the nanoscale dynamic organization of ion channels in cell membranes**. *Sci Rep* 2017, **7**:16647.

4. • Freeman SA, Vega A, Riedl M, Collins RF, Ostrowski PP, Woods EC, Bertozzi CR, Tammi MI, Lidke DS, Johnson P *et al.*: **Transmembrane pickets connect cyto- and pericellular**

skeletons forming barriers to receptor engagement. *Cell* 2018, **172**:305-317 e10.
The fences and pickets model of plasma membrane organization was proposed in 2002. This work identified the first known picket protein, CD44.

5. Bausch-Fluck D, Hofmann A, Bock T, Frei AP, Cerciello F, Jacobs A, Moest H, Omasits U, Gundry RL, Yoon C *et al.*: **A mass spectrometric-derived cell surface protein atlas**. *PLoS One* 2015, **10**e0121314.

6. Dustin ML, Groves JT: **Receptor signaling clusters in the immune synapse**. *Annu Rev Biophys* 2012, **41**:543-556.

7. Lee K-H, Dinner AR, Tu C, Campi G, Raychaudhuri S, Varma R, Sims TN, Burack WR, Wu H, Wang J *et al.*: **The immunological synapse balances T cell receptor signaling and degradation**. *Science* 2003, **302**:1218-1222.

8. • Hay DL, Pioszak AA: **Receptor activity-modifying proteins (RAMPs): new insights and roles**. *Annu Rev Pharmacol Toxicol* 2016, **56**:469-487.
Review of modulatory actions of receptor activity-modifying proteins (RAMPs) on GPCR function and signaling.

9. Termini CM, Gillette JM: **Tetraspanins function as regulators of cellular signaling**. *Front Cell Dev Biol* 2017, **5**:34.

10. Charrin S, le Naour F, Silvie O, Milhiet P-E, Boucheix C, Rubinstein E: **Lateral organization of membrane proteins: tetraspanins spin their web**. *Biochem J* 2009, **420**:133-154.

11. Zona L, Tawar RG, Zeisel MB, Xiao F, Schuster C, Lupberger J, Baumert TF: **CD81-receptor associations–impact for hepatitis C virus entry and antiviral therapies**. *Viruses* 2014, **6**:875-892.

12. Zaitseva M, Romantseva T, Manischewitz J, Wang J, Goucher D, Golding H: **Increased CXCR4-dependent HIV-1 fusion in activated T cells: role of CD4/CXCR4 association**. *J Leukoc Biol* 2005, **78**:1306-1317.

13. Xiao X, Wu L, Stantchev TS, Feng YR, Ugolini S, Chen H, Shen Z, Riley JL, Broder CC, Sattentau QJ *et al.*: **Constitutive cell surface association between CD4 and CCR5**. *Proc Natl Acad Sci U S A* 1999, **96**:7496-7501.

14. •• Nussinov R, Jang H, C-J Tsai: **Oligomerization and nanocluster organization render specificity**. *Biol Rev Camb Philos Soc* 2015, **90**:587-598.
Review of the reasons for and consequences of oligomerization and nanocluster formation.

15. Chylek LA, Akimov V, Dengjel J, Rigbolt KTG, Hu B, Hlavacek WS, Blagoev B: **Phosphorylation site dynamics of early T-cell receptor signaling**. *PLoS One* 2014, **9**e104240.

16. Huttlin EL, Ting L, Bruckner RJ, Gebreab F, Gygi MP, Szpyt J, Tam S, Zarraga G, Colby G, Baltier K *et al.*: **The BioPlex network: a systematic exploration of the human interactome**. *Cell* 2015, **162**:425-440.

17. Hein MY, Hubner NC, Poser I, Cox J, Nagaraj N, Toyoda Y, Gak IA, Weisswange I, Mansfeld J, Buchholz F *et al.*: **A human interactome in three quantitative dimensions organized by stoichiometries and abundances**. *Cell* 2015, **163**:712-723.

18. Rolland T, Taşan M, Charloteaux B, Pevzner SJ, Zhong Q, Sahni N, Yi S, Lemmens I, Fontanillo C, Mosca R *et al.*: **A proteome-scale map of the human interactome network**. *Cell* 2014, **159**:1212-1226.

19. Titeca K, Lemmens I, Tavernier J, Eyckerman S: **Discovering cellular protein-protein interactions: technological strategies and opportunities**. *Mass Spectrom Rev* 2018 http://dx.doi.org/10.1002/mas.21574.

20. Bushell KM, Söllner C, Schuster-Boeckler B, Bateman A, Wright GJ: **Large-scale screening for novel low-affinity extracellular protein interactions**. *Genome Res* 2008, **18**:622-630.

21. Frei AP, Jeon O-Y, Kilcher S, Moest H, Henning LM, Jost C, Plückthun A, Mercer J, Aebersold R, Carreira EM *et al.*: **Direct identification of ligand-receptor interactions on living cells and tissues**. *Nat Biotechnol* 2012, **30**:997-1001.

22. Sobotzki N, Schafroth MA, Rudnicka A, Kötemann A, Marty F, Götze S, Yamauchi Y, Carreira EM, Wollscheid B: **HATRIC-based identification of receptors for orphan ligands**. *Nat Commun* 2018.

23. Peled M, Tocheva AS, Sandigursky S, Nayak S, Philips EA, Nichols KE, Strazza M, Azoulay-Alfaguter I, Askenazi M, Neel BG *et al.*: **Affinity purification mass spectrometry analysis of PD-1 uncovers SAP as a new checkpoint inhibitor**. *Proc Natl Acad Sci U S A* 2018, **115**:E468-E477.

24. Daulat AM, Maurice P, Jockers R: **Tandem affinity purification and identification of GPCR-associated protein complexes**. *Methods Mol Biol* 2011, **746**:399-409.

25. Savas JN, Stein BD, Wu CC, Yates JR 3rd: **Mass spectrometry accelerates membrane protein analysis**. *Trends Biochem Sci* 2011, **36**:388-396.

26. • Zhang Q, Park E, Kani K, Landgraf R: **Functional isolation of activated and unilaterally phosphorylated heterodimers of ERBB2 and ERBB3 as scaffolds in ligand-dependent signaling**. *Proc Natl Acad Sci U S A* 2012, **109**:13237-13242.
Work dissecting the stoichiometry of ERBB2/ERBB3 complexes and demonstrating that the stoichiometry is dependent on the relative abundances of ERBB2 and ERBB3.

27. Roux KJ, Kim DI, Raida M, Burke B: **A promiscuous biotin ligase fusion protein identifies proximal and interacting proteins in mammalian cells**. *J Cell Biol* 2012, **196**:801-810.

28. Lam SS, Martell JD, Kamer KJ, Deerinck TJ, Ellisman MH, Mootha VK, Ting AY: **Directed evolution of APEX2 for electron microscopy and proximity labeling**. *Nat Methods* 2015, **12**:51-54.

29. Jiang S, Kotani N, Ohnishi T, Miyagawa-Yamguchi A, Tsuda M, Yamashita R, Ishiura Y, Honke K: **A proteomics approach to the cell-surface interactome using the enzyme-mediated activation of radical sources reaction**. *Proteomics* 2012, **12**:54-62.

30. Martell JD, Yamagata M, Deerinck TJ, Phan S, Kwa CG, Ellisman MH, Sanes JR, Ting AY: **A split horseradish peroxidase for the detection of intercellular protein-protein interactions and sensitive visualization of synapses**. *Nat Biotechnol* 2016, **34**:774-780.

31. Rees JS, Li X-W, Perrett S, Lilley KS, Jackson AP: **Selective proteomic proximity labeling assay using tyramide (SPPLAT): a quantitative method for the proteomic analysis of localized membrane-bound protein clusters**. *Curr Protoc Protein Sci* 2015.

32. Bar DZ, Atkatsh K, Tavarez U, Erdos MR, Gruenbaum Y, Collins FS: **Biotinylation by antibody recognition–a method for proximity labeling**. *Nat Methods* 2017, **15**:127.

33. Koos B, Andersson L, Clausson C-M, Grannas K, Klaesson A, Cane G, Söderberg O: **Analysis of protein interactions in situ by proximity ligation assays**. *Curr Top Microbiol Immunol* 2014, **377**:111-126.

34. Ghosh I, Hamilton AD, Regan L: **Antiparallel leucine zipper-directed protein reassembly: application to the green fluorescent protein**. *J Am Chem Soc* 2000, **122**:5658-5659.

35. Filonov GS, Verkhusha VV: **A near-infrared BiFC reporter for in vivo imaging of protein-protein interactions**. *Chem Biol* 2013, **20**:1078-1086.

36. Gordon GW, Berry G, Liang XH, Levine B, Herman B: **Quantitative fluorescence resonance energy transfer measurements using fluorescence microscopy**. *Biophys J* 1998, **74**:2702-2713.

37. Pfleger KDG, Seeber RM, Eidne KA: **Bioluminescence resonance energy transfer (BRET) for the real-time detection of protein-protein interactions**. *Nat Protoc* 2006, **1**:337-345.

38. Petschnigg J, Groisman B, Kotlyar M, Taipale M, Zheng Y, Kurat CF, Sayad A, Sierra JR, Mattiazzi Usaj M, Snider J *et al.*: **The mammalian-membrane two-hybrid assay (MaMTH) for probing membrane-protein interactions in human cells**. *Nat Methods* 2014, **11**:585-592.

39. Pu J, Zinkus-Boltz J, Dickinson BC: **Evolution of a split RNA polymerase as a versatile biosensor platform**. *Nat Chem Biol* 2017, **13**:432-438.

40. Wehr MC, Laage R, Bolz U, Fischer TM, Grünewald S, Scheek S, Bach A, Nave K-A, Rossner MJ: **Monitoring regulated protein-protein interactions using split TEV**. *Nat Methods* 2006, **3**:985-993.

41. Leng W, Pang X, Xia H, Li M, Chen L, Tang Q, Yuan D, Li R, Li L, Gao F *et al.*: **Novel split-luciferase-based genetically encoded biosensors for noninvasive visualization of Rho GTPases**. *PLoS One* 2013, **8**e62230.

42. Galarneau A, Primeau M, Trudeau L-E, Michnick SW: **β-Lactamase protein fragment complementation assays as in vivo and in vitro sensors of protein–protein interactions**. *Nat Biotechnol* 2002, **20**:619.

43. Wiens MD, Campbell RE: **Surveying the landscape of optogenetic methods for detection of protein-protein interactions**. *Wiley Interdiscip Rev Syst Biol Med* 2018 http://dx.doi.org/10.1002/wsbm.1415.

44. Snider J, Kotlyar M, Saraon P, Yao Z, Jurisica I, Stagljar I: **Fundamentals of protein interaction network mapping**. *Mol Syst Biol* 2015, **11**:848.

45. Dunham WH, Mullin M, A-C Gingras: **Affinity-purification coupled to mass spectrometry: Basic principles and strategies**. *Proteomics* 2012, **12**:1576-1590.

46. Selbach M, Mann M: **Protein interaction screening by quantitative immunoprecipitation combined with knockdown (QUICK)**. *Nat Methods* 2006, **3**:981-983.

47. Branon TC, Bosch JA, Sanchez AD, Udeshi ND: **Directed evolution of TurboID for efficient proximity labeling in living cells and organisms**. *bioRxiv* 2017.

48. Schopp IM, Amaya Ramirez CC, Debeljak J, Kreibich E, Skribbe M, Wild K, Béthune J: **Split-BioID a conditional proteomics approach to monitor the composition of spatiotemporally defined protein complexes**. *Nat Commun* 2017, **8**:15690.

49. •• Toscani AM, Sampayo RG, Barabas FM, Fuentes F, Simian M, Leskow FC: **Distinct ErbB2 receptor populations differentially interact with beta1 integrin in breast cancer cell models**. *PLoS One* 2017, **12**e0174230.
Elegant demonstration that the abundance of ErbB2 receptor influences its lateral plasma membrane interactions.

50. • Washburn MP: **There is no human interactome**. *Genome Biol* 2016, **17**:48.
Review summarizing current knowledge of interaction promiscuity specifically focused on the mediator complex and how post-translational modifications influence the type of interaction and thus architecture of the complex.

51. • Fujiwara T, Ritchie K, Murakoshi H, Jacobson K, Kusumi A: **Phospholipids undergo hop diffusion in compartmentalized cell membrane**. *J Cell Biol* 2002, **157**:1071-1081.
Superfast single-molecule tracking of phospholipids revealed a specific diffusion pattern, named hop diffusion, and led to proposal of the fences and pickets model.

52. Simons K, Toomre D: **Lipid rafts and signal transduction**. *Nat Rev Mol Cell Biol* 2000, **1**:31-39.

53. • Casaletto JB, McClatchey AI: **Spatial regulation of receptor tyrosine kinases in development and cancer**. *Nat Rev Cancer* 2012, **12**:387-400.
Interesting review that discusses the influence of RTK abundance and how its lateral (within the plasma membrane) and axial (endocytosis) spatial regulation influence signaling strength

54. •• Sungkaworn T, Jobin M-L, Burnecki K, Weron A, Lohse MJ, Calebiro D: **Single-molecule imaging reveals receptor–G protein interactions at cell surface hot spots**. *Nature* 2017, **550**nature24264.
Revolutionary work in which simultaneous single-molecule tracking of a GPCR and its interacting G-protein and of the transition from G_i to the activated G_s molecule revealed that the interaction between the GPCR and its G-protein occurs in defined regions of the plasma membrane and that the location also influences the activation of G_s.

55. Chung I: **Optical measurement of receptor tyrosine kinase oligomerization on live cells**. *Biochim Biophys Acta* 2017, **1859**:1436-1444.

56. Smith LM, Kelleher NL: **Consortium for top down proteomics: proteoform: a single term describing protein complexity**. *Nat Methods* 2013, **10**:186-187.

57. Pinho SS, Reis CA: **Glycosylation in cancer: mechanisms and clinical implications**. *Nat Rev Cancer* 2015, **15**:540-555.

58. Fuster MM, Esko JD: **The sweet and sour of cancer: glycans as novel therapeutic targets**. *Nat Rev Cancer* 2005, **5**:526-542.

59. • Kaszuba K, Grzybek M, Orłowski A, Danne R, Róg T, Simons K, Coskun Ü, Vattulainen I: **N-Glycosylation as determinant of epidermal growth factor receptor conformation in membranes**. *Proc Natl Acad Sci U S A* 2015, **112**:4334-4339.
Article that demonstrate how glycosylation influences the conformation of EGFR and thus its functionality.

60. Soderquist AM, Carpenter G: **Glycosylation of the epidermal growth factor receptor in A-431 cells. The contribution of carbohydrate to receptor function**. *J Biol Chem* 1984, **259**:12586-12594.

61. Cai X, Thinn AMM, Wang Z, Shan H, Zhu J: **The importance of N-glycosylation on β3 integrin ligand binding and conformational regulation**. *Sci Rep* 2017, **7**.

62. Blidner AG, Méndez-Huergo SP, Cagnoni AJ, Rabinovich GA: **Re-wiring regulatory cell networks in immunity by galectin-glycan interactions**. *FEBS Lett* 2015, **589**:3407-3418.

63. Rabinovich GA, Croci DO: **Regulatory circuits mediated by lectin-glycan interactions in autoimmunity and cancer**. *Immunity* 2012, **36**:322-335.

64. • Kennedy SP, Hastings JF, Han JZR, Croucher DR: **The under-appreciated promiscuity of the epidermal growth factor receptor family**. *Front Cell Dev Biol* 2016, **4**:88.
Review that summarized the large number of lateral interaction partners of EGFR.

65. Lee H-W, Choi B, Kang HN, Kim H, Min A, Cha M, Ryu JY, Park S, Sohn J, Shin K *et al.*: **Profiling of protein–protein interactions via single-molecule techniques predicts the dependence of cancers on growth-factor receptors**. *Nature Biomedical Engineering* 2018 http://dx.doi.org/10.1038/s41551-018-0212-3.

66. Cuesta AM, Sainz-Pastor N, Bonet J, Oliva B, Alvarez-Vallina L: **Multivalent antibodies: when design surpasses evolution**. *Trends Biotechnol* 2010, **28**:355-362.

67. Chang C-H, Wang Y, Li R, Rossi DL, Liu D, Rossi EA, Cardillo TM, Goldenberg DM: **Combination therapy with bispecific antibodies and PD-1 blockade enhances the antitumor potency of T cells**. *Cancer Res* 2017, **77**:5384-5394.

68. Marshall DJ, Harried SS, Murphy JL, Hall CA, Shekhani MS, Pain C, Lyons CA, Chillemi A, Malavasi F, Pearce HL *et al.*: **Extracellular antibody drug conjugates exploiting the proximity of two proteins**. *Mol Ther* 2016, **24**:1760-1770.

69. • Hopkins AL: **Network pharmacology: the next paradigm in drug discovery**. *Nat Chem Biol* 2008, **4**:682-690.
Since single effector molecules targeting single receptors have low efficacy and provoke resistance, new combinatorial approaches are needed. This review discusses the opportunities and challenges of polypharmacology or network pharmacology.

70. Bernardino de la Serna J, Schütz GJ, Eggeling C, Cebecauer M: **There is no simple model of the plasma membrane organization**. *Front Cell Dev Biol* 2016, **4**:106.

71. Liu Q, Zheng J, Sun W, Huo Y, Zhang L, Hao P, Wang H, Zhuang M: **A proximity-tagging system to identify membrane protein–protein interactions**. *Nat Methods* 2018, **15**.