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Trends in
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Forum

Validation of agent-based models of surface receptor oligomerisation

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Receptor dimerisation and higher order oligomerisation regulates signalling by a wide variety of transmembrane receptors. We discuss how agent-based modelling (ABM) combined with advanced microscopy and structural studies can provide new insights into the regulation of clustering, including spatial considerations, revealing novel targets for therapeutic intervention.

Historical perspective of modelling receptor clustering

The oligomerisation of cell surface proteins in the plasma membrane has been an area of great interest and intense research for decades. The importance of receptor oligomers, such as dimers, particularly with single transmembrane receptors, in modulating activation and signalling is well established. However, until recently, the study of **receptor clustering** (see Glossary) at the nanoscale and the regulation of the biological processes of oligomerisation has remained oversimplified and poorly understood. Recent advances in microscopy, modelling, and structural studies provide an opportunity to utilise a combined approach to generate and test hypotheses in relation to receptor clustering.

The binding of a monovalent ligand to a receptor has been modelled according to the **law of mass action** using **ordinary differential equations (ODEs)** to provide fundamental theories and conclusions on the process of ligand engagement and receptor activation [1]. The law of mass action describes receptor occupancy but does not take into consideration the conformational changes of the receptor that lead to activation, such as with G protein-coupled receptors (GPCRs) and ion channels. This was addressed by the development of the two-state model and later by more complex models to account for features such as efficacy, inverse agonism, and **receptor desensitisation** [1]. The modelling of single transmembrane receptors such as a receptor tyrosine kinase using ODEs has shown that activation can occur exclusively by receptor dimerisation/oligomerisation and independent of intracellular kinase activation or a change in receptor conformation [1], highlighting the importance of receptor oligomerisation. However, the addition of features such as receptor homodimerisation and association of intracellular tails with signalling proteins results in a more complex model which cannot be readily modelled using ODE and consequently its wider application in biological processes are limited. Furthermore, ODE modelling cannot provide spatial considerations which is critical when investigating how the distribution of a receptor changes following ligand binding and activation. This missing information can be combined with structural and single-molecule microscopy studies to identify novel sites or mechanisms for the development of therapeutics (Table 1).

ABM: an alternative approach?

The limitations of ODE modelling to model receptor oligomerisation with higher levels of complexity means other methods are needed. ABM offers an alternative approach which has several advantages and can also include spatial considerations. ABM is a computational approach that generates **stochastic simulations** which explore the behaviour of autonomous

Glossary

Expansion microscopy (ExM): a sample preparation technique used in the field of microscopy to enhance the resolution of imaging biological samples below the diffraction limit of light (<200 nm). It physically expands samples using a polymer system while maintaining the samples, structural integrity.
Fluorescence correlation spectroscopy (FCS): a powerful technique used in biophysics and cell biology to study the dynamics of fluorescently tagged proteins within a stationary small volume (typically femtoliters). It monitors fluorescent fluctuations to generate quantitative information about molecular diffusion, molecular brightness, binding interactions, and concentrations.
Law of mass action: a fundamental concept that describes the relationship between the concentrations of the reactants and the products in a chemical reaction at equilibrium. The law states that the rate of any chemical reaction is proportional to the concentrations of the reactants and at equilibrium, the ratio between the reactant and product concentrations will be constant.
Ordinary differential equations (ODEs): a type of differential equation that involves a single independent variable and one or more dependent variables. They represent relationships between the rates of change of a function and the function itself. They describe how a function and its derivatives vary with respect to the independent variable.
Receptor clustering: a mechanism where cell surface proteins such as membrane receptors bind and group together to form clusters or aggregates in specific regions of the cell membrane. The localised clusters are formed by the redistribution and accumulation of receptors through various mechanisms including direct protein–protein interactions such as homodimerisation, ligand binding, and interactions with other membrane proteins. Clustering contributes to the regulation and efficiency of cellular signalling.
Receptor desensitisation: a regulatory mechanism by which cells reduce their responsiveness following continuous or prolonged stimulation by a ligand, such as hormones, neurotransmitters, growth factors, or therapeutics. This occurs by protein internalisation and downregulation or activation of inhibitory pathways and is important in maintaining cellular homeostasis.
Single-molecule localisation microscopy (SMLM): a group of powerful advanced imaging techniques that significantly improves spatial resolution compared with diffraction limited optical techniques. It enables visualisation and precise localisation of molecules with nanometer-scale resolution, generating super-resolution images of cellular and molecular structures such as receptors. Examples include stochastic optical reconstruction microscopy (dSTORM) and photoactivated localisation microscopy (PALM).
Single particle tracking (SPT): a tool used in microscopy to monitor the movements of individual

particles or molecules within a medium or in living cells. It is able to follow or track the motion of particles over time to generate trajectories which can provide insights into the dynamics, behaviour, and interactions of particles at a single-molecule level.
Stochastic simulations: a computational technique used to model and analyse systems that involve randomness or uncertainty. The simulation variables may change stochastically (randomly) with individual probabilities and may produce different outputs from the same starting condition.
Total internal reflection fluorescence (TIRF) microscopy: an optical imaging technique used to visualise events near the surface of a sample with high signal-to-noise ratio, improved axial resolution, and reduced photobleaching and phototoxicity compared with conventional widefield fluorescence microscopy. It exploits the properties of total internal reflection which occurs when light travels through two mediums with different refractive indices. When light travelling through a medium encounters a medium with a lower refractive index and is above a particular incident angle, the light is reflected creating an evanescent wave which penetrates a short distance (100–200 nM) of the sample.

agents under a defined set of rules and is well suited for simulation of biological systems whose properties are usually only partially known [1] (Figure 1). In particular, ABM can help to identify key regulatory steps in a complex system without a full understanding of all the components. In addition, ABM allows spatial relationships to be considered, which is difficult to achieve with equation-based methods [1]. Thus, in ABM, it is relatively easy to integrate conditional pre-set rules that dynamically change the state of each receptor based on location as well as changes such as phosphorylation, homodimerisation, and interactions with other membrane proteins and intracellular signalling molecules [1–3] (Figure 1). For example, ABM has been used to monitor the nanoscale clustering of surface proteins based on a 'desire for clustering' parameter that represents several competing cellular processes that govern protein distribution, including self-affinity, membrane lipid domains, and cortical actin [4]. ABM has also been used to aid the understanding of key determinants of receptor diffusion and signalling in lipid rafts and to identify receptor density, surface area, confinement, and additional proteins as variables that influence receptor dimerisation and higher order organisation [5]. ABM can, therefore, be used to generate hypotheses for testing in wet-laboratory experiments using **single-molecule localisation microscopy (SMLM)** and advanced microscopy methods that provide coordinate data on positions, movements, and affinities. When combined with structural data which yields information on sites of interaction including key amino acid residues that support dimerisation and higher order clustering, this can aid the development of novel therapeutics.

Table 1. Examples of the implications of using a combinational approach of ABM, structural studies, and advanced microscopy to investigate receptor oligomerisation for drug discovery and development

Example spatio-temporal regulatory outputs from ABM and advanced microscopy	Examples	Implications for drug discovery and development
Determination of receptor stoichiometry such as dimerisation	• CLEC-2 [8] • β_1/β_2 adrenoceptors and $GABA_B$ GPCRs [12]	Potential new sites to target
Blocking dimerisation	• GPVI blocker glenzocimab [14]	Inhibiting activation by preventing dimerisation and/or ligand binding
Modelling and mapping sites of interaction with other membrane proteins	• $GABA_B$ receptor and cytoskeleton/filamin A [12,15]	New sites to target protein localisation, trafficking, and interactions with regulatory proteins
Identification of multiple mechanisms of receptor dimerisation/oligomerisation	• Class I cytokine receptors and JAK2 [16] • c-Mpl and thrombopoietin [17]	Potential new strategy of using combinational therapy with lower doses and reduced off-target effects
Mapping of receptor oligomerisation following engagement with a multivalent ligand	• GPVI [9,10] • CLEC-2 [8]	The need for development of a high-affinity blocker or combinational therapy with a weak blocker of a signalling molecule to prevent receptor oligomerisation
Determination of kinetic data and affinities of protein–protein interactions	• α_{2A}-adrenoceptor and $G\alpha_i$ protein [11] • Neurotensin receptor 1 [18]	A critical regulator of drug efficacy

Combining ABM with advanced microscopy: a powerful approach to investigate protein clustering

With recent advancements in super-resolution single-molecule microscopy and analysis platforms, the study of oligomerisation and the nanoscale clustering of proteins including membrane receptors has become more accessible, and easier to perform, analyse, and interpret. Here, we discuss a selection of techniques that could be used in combination with ABM to address key specific questions revolving around the oligomerisation of proteins, namely **fluorescence correlation spectroscopy (FCS)**, SMLM, **single particle tracking (SPT),** and **expansion microscopy (ExM)**.

The single-molecule technique of FCS has come to the forefront as a powerful technique to investigate protein diffusion and protein–protein interactions in the membrane of live cells. FCS monitors fluorescent fluctuations generated by mobile fluorescently tagged proteins diffusing through a stationary confocal volume (~0.2 fl) in a temporal manner to generate information about their diffusion and brightness. By utilising the molecular brightness measurements with control proteins, it can be used to measure

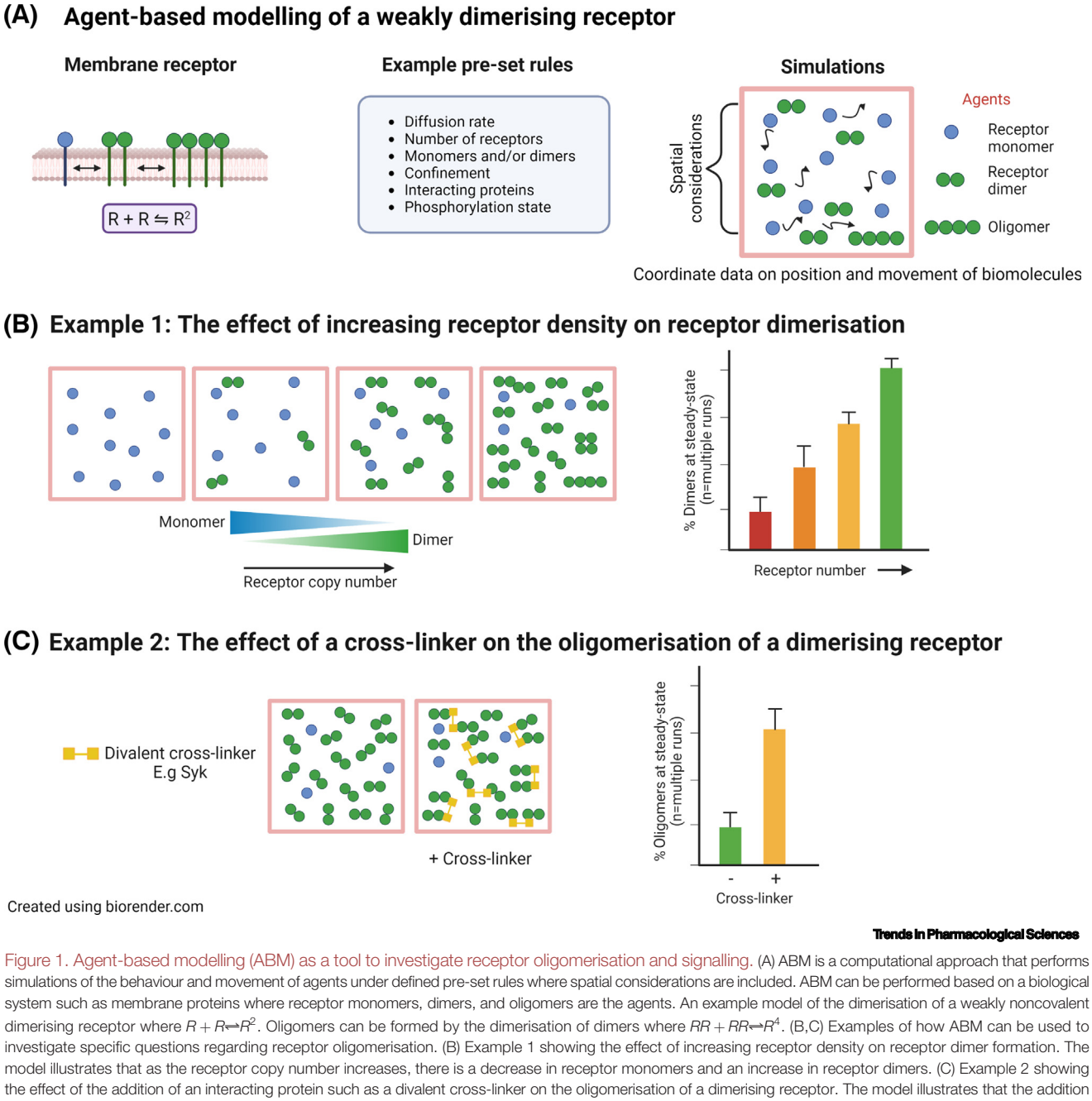

Figure 1. Agent-based modelling (ABM) as a tool to investigate receptor oligomerisation and signalling. (A) ABM is a computational approach that performs simulations of the behaviour and movement of agents under defined pre-set rules where spatial considerations are included. ABM can be performed based on a biological system such as membrane proteins where receptor monomers, dimers, and oligomers are the agents. An example model of the dimerisation of a weakly noncovalent dimerising receptor where $R + R \rightleftharpoons R^2$. Oligomers can be formed by the dimerisation of dimers where $RR + RR \rightleftharpoons R^4$. (B,C) Examples of how ABM can be used to investigate specific questions regarding receptor oligomerisation. (B) Example 1 showing the effect of increasing receptor density on receptor dimer formation. The model illustrates that as the receptor copy number increases, there is a decrease in receptor monomers and an increase in receptor dimers. (C) Example 2 showing the effect of the addition of an interacting protein such as a divalent cross-linker on the oligomerisation of a dimerising receptor. The model illustrates that the addition of a cross-linker such as the signalling kinase Syk drives an increase in the oligomerisation of a dimerising receptor such as CLEC-2.

the dimerisation and higher order oligomerisation of proteins [6]. For example, we have shown the platelet glycoprotein receptors, glycoprotein VI (GPVI), and C-type lectin-like receptor (CLEC-2) are expressed as a dynamic mixture of monomers and dimers [7,8] with increased dimerisation observed following ligand addition or increased receptor expression. We have been able to model and test other parameters that regulate clustering as exemplified by the contribution of the tandem SH2 domain kinase Syk, to clustering of CLEC-2 [1]. This illustrates how ABM and microscopy can be combined to model and test new hypotheses on protein organisation.

The nanoscale spatial organisation of membrane proteins has also been studied by SMLM which utilises the ability of fluorescent molecules to switch between on (fluorescent) and off (dark) states (referred to as blinking) to estimate the positions of molecules below the diffraction limit of light to a distance in the order of 10 nm. The optical imaging technique of **total internal reflection fluorescence (TIRF) microscopy** is used in SMLM to visualise molecules near the surface of a sample (100–200 nM axial depth) with high signal-to-noise ratio, improved resolution, and reduced sample destruction. SMLM has been used to image GPVI clustering along collagen fibres in platelets and to show how this leads to spatial separation from the sheddase ADAM10 [9,10]. SPT also uses TIRF microscopy to visualise the movement of proteins in live cells with high spatial (~20 nm) and temporal (~30 ms) resolution enabling trajectories of protein diffusion to be generated. It can be used to determine colocalisation and kinetic information of protein–protein interactions by monitoring on- and off-rates of interactions and thus enables the K_d to be determined as exemplified by the interaction of adrenoceptors and G proteins [11]. Furthermore, it has been used to investigate the supramolecular organisation and mobility of the prototypical β_1/β_2 adrenoceptors and $GABA_B$ GPCRs [12]. Together with structural studies which can identify interaction surfaces on proteins, it is possible to map critical interactions and mechanisms.

The relatively new microscopy technique of ExM is based on the physical volumetric expansion of a sample and can be combined with other super-resolution methods such as SMLM to deliver unprecedented resolution. For example, it has been used to investigate the organisation of the highly abundant integrin αIIbβ3 on platelets [13]. This would not have been easily performed with other super-resolution techniques due to its high density. ExM, therefore, provides the testing of ABMs of highly expressed proteins where other microscopy techniques would not be suitable.

Concluding remarks and future perspectives

Cutting-edge advanced microscopy techniques together with structural studies could provide a useful way of biologically testing focused and specific questions from models generated through ABM to enable a deeper understanding of the highly controlled regulation of cellular protein organisation and how this relates to receptor activation and signalling (see Table 1 for examples). We propose that this combinational approach will be a powerful tool to develop the complexity of the spatio-temporal regulation of receptor clustering and stimulation of signalling pathways and that this can be applied to the development of therapeutics with improved efficacy.

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Declaration of interests

The authors declare no conflicts of interest.

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References

1. Maqsood, Z. *et al.* (2022) Experimental validation of computerised models of clustering of platelet glycoprotein receptors that signal via tandem SH2 domain proteins. *PLoS Comput. Biol.* 18, e1010708
2. Das, A.A. *et al.* (2017) Agent-based re-engineering of ErbB signaling: a modeling pipeline for integrative systems biology. *Bioinformatics* 33, 726–732
3. Soheilypour, M. and Mofrad, M.R.K. (2018) Agent-based modeling in molecular systems biology. *BioEssays* 40, e1800020
4. Griffié, J. *et al.* (2020) An agent-based model of molecular aggregation at the cell membrane. *PLoS One* 15, e0226825
5. Tantiwong, C. *et al.* (2023) An agent-based approach for modelling and simulation of glycoprotein VI receptor diffusion, localisation and dimerisation in platelet lipid rafts. *Sci. Rep.* 13, 3906
6. Horsey, A.J. *et al.* (2020) Application of fluorescence correlation spectroscopy to study substrate binding in styrene maleic acid lipid copolymer encapsulated ABCG2. *Biochim. Biophys. Acta Biomembr.* 1862, 183218
7. Clark, J.C. *et al.* (2021) Evidence that GPVI is expressed as a mixture of monomers and dimers, and that the D2 domain is not essential for GPVI activation. *Thromb. Haemost.* 121, 1435–1447
8. Clark, J.C. *et al.* (2023) Divalent nanobodies to platelet CLEC-2 can serve as agonists or antagonists. *Commun. Biol.* 6, 376
9. Poulter, N.S. *et al.* (2017) Clustering of glycoprotein VI (GPVI) dimers upon adhesion to collagen as a mechanism to regulate GPVI signaling in platelets. *J. Thromb. Haemost.* 15, 549–564
10. Pallini, C. *et al.* (2021) Immobilized collagen prevents shedding and induces sustained GPVI clustering and signaling in platelets. *Platelets* 32, 59–73
11. Sungkaworn, T. *et al.* (2017) Single-molecule imaging reveals receptor-G protein interactions at cell surface hot spots. *Nature.* 550, 543–547
12. Calebiro, D. *et al.* (2013) Single-molecule analysis of fluorescently labeled G-protein-coupled receptors reveals complexes with distinct dynamics and organization. *Proc. Natl. Acad. Sci. U. S. A.* 110, 743–748
13. Heil, H.S. *et al.* (2022) Mapping densely packed αIIbβ3 receptors in murine blood platelets with expansion microscopy. *Platelets* 33, 849–858
14. Billiald, P. *et al.* (2023) Targeting platelet GPVI with glenzocimab: a novel mechanism for inhibition. *Blood Adv.* 7, 1258–1268
15. Jobin, M.L. *et al.* (2023) Filamin A organizes γ-aminobutyric acid type B receptors at the plasma membrane. *Nat. Commun.* 14, 34
16. Wilmes, S. *et al.* (2020) Mechanism of homodimeric cytokine receptor activation and dysregulation by oncogenic mutations. *Science* 367, 643–652
17. Hitchcock, I.S. *et al.* (2021) The thrombopoietin receptor: revisiting the master regulator of platelet production. *Platelets* 32, 770–778
18. Dijkman, P.M. *et al.* (2018) Dynamic tuneable G protein-coupled receptor monomer-dimer populations. *Nat. Commun.* 9, 1710