**2. Stochastic model of receptor diffusion, interactions and sub-stoichiometric labeling**

To test the feasibility of FISIK, we employed a simple stochastic model of receptor diffusion and interactions as well as sub-stoichiometric labeling to mimic single-molecule sampling. In this model, receptors have a surface density ρ, undergo 2D free diffusion with diffusion coefficient D, and can form oligomers up to maximum oligomer size Omax. In the following and subsequent descriptions, oligomer(n) indicates an oligomer of size n, where n=1 means monomer, n=2 means dimer, n=3 means trimer, etc. The terms monomer and oligomer(1) will be used interchangeably, as needed for compactness of description. When a receptor and an oligomer(n≥1) approach each other within the association distance (dassoc; taken as 10 nm), they associate with probability pa(n+1) to form an oligomer(n+1). The case of n = 1 is the special case of two monomers associating to form a dimer. Note that in this model, for the sake of simplicity, association and dissociation are limited to one receptor at a time. Receptors in an oligomer diffuse together, with the same diffusion coefficient D. For an oligomer(n≥2), a receptor can dissociate from it with rate koff(n), to produce a monomer and an oligomer(n-1). To mimic single-molecule imaging, a fraction f of the receptors is labeled, with individual fluorophore intensity I ~ N(μi,σi) (i.e. the intensity of an individual fluorophore fluctuates over time, mimicking realistic fluorophore intensity fluctuations). Receptor trajectories and interactions are generated from the model using kinetic Monte Carlo simulations (described in subsection 3 below), and then the trajectories and interactions of the labeled subset are output.

The unknown parameters in this model are the receptor motion parameter D, the receptor interaction parameters pa(n) and koff(n) (n=2, 3, 4, …), the receptor population parameters ρ and f, and the fluorophore intensity parameters μi and σi. As mentioned above, D can be estimated directly from the single-molecule data, as it does not suffer from the undersampling issue. The fluorophore intensity parameters can be estimated experimentally as well. The other parameters, however, must be estimated by fitting the model to experimental data, as described in the general framework above (subsection 1), and using the intermediate statistics described in subsection 4 below.

**3. Kinetic Monte Carlo simulations**

To generate data from the above model, we used kinetic Monte Carlo simulations explicit in both space and time. In these simulations, space is treated as a continuum, while time is discretized into a series of time steps Δt, where Δt is small enough to minimize discretization artifacts (as described in detail below). To simulate receptors at density ρ within a simulation area A, Nrec = ρ × A receptors are initially placed randomly within the simulation area, all starting as monomers. Reflecting boundary conditions are used, and the number of receptors is kept constant during the whole simulation. Each simulation starts with an initialization time Tinit (usually 10 s) so that the system reaches steady state (S1 Fig), followed by the desired simulation time Tsim. Only the steady state trajectories and interactions are output for further processing and analysis.

At every time point t after the initial time point, receptors can dissociate, move and associate, in this order, as described in the following:

(1) Receptor dissociation: For each oligomer(n ≥ 2) at the previous time point (t – Δt), one receptor can dissociate from it at time point t with probability poff(n) = koff(n) × Δt, producing a monomer and an oligomer(n-1). If a dissociation happens, the involved monomer and oligomer are not allowed to associate with each other or with any other receptor or oligomer in the current time point, to avoid what will appear as simultaneous swapping of receptors between oligomers in one time point. This constraint is acceptable as long as the time step is small enough.

(2) Initial update of receptor positions: Under the model of free diffusion, each oligomer(n ≥ 1) takes a step from time point t – Δt to time point t with x- and y-components, sx and sy, ~ , where D is the diffusion coefficient. As mentioned above, all receptors within an oligomer move together. Note that if a receptor has dissociated from an oligomer (point 1 above), it moves independently of the oligomer to which it used to belong. The resulting positions are considered “initial positions” because, as described next, these positions might be altered by association.

(3) Receptor association and final update of receptor positions: Due to time discretization in the simulations, receptor encounters, a prerequisite for association, must be handled in a special way. The reason is that receptors might pass by each other as they move from one time point to the next, but might not be closer than dassoc (= 10 nm) at either time point. Without taking this issue into account, the number of detected receptor encounters, and thus the rate of receptor association, decreases as Δt increases (S2 Fig). However, simulating the system with Δt < 0.001-0.01 is computationally prohibitive. Therefore, we devised a simulation strategy to compensate for the effect of finite Δt on receptor encounters, rendering the simulation output insensitive to Δt upto 0.01 s, a practically feasible simulation time step (S2 Fig). The compensation strategy is based on assigning molecules an “effective radius” (Reff) based on their extent of movement within Δt, defined as . With this, an effective association distance is then used in the simulations, defined as dassoc,eff = max(2Reff, dassoc). In other words, as long as the scale of movement within one time step is larger than the scale of molecular size, the scale of movement determines the effective association distance.

Using dassoc,eff, all monomer-oligomer(n ≥ 1) pairs with pairwise distance ≤ dassoc,eff are considered as encounter candidates, with the actual pairs undergoing encounters determined by graph matching. Monomer-oligomer(n ≥ 1) pairs that encounter each other might associate with each other with probability pa(n+1) (an input model parameter) to form an oligomer(n+1). When an association happens, the position of the resulting oligomer is the average position of the two associating entities. This yields the final positions of receptors at time t of the simulation.