Supplementary Information

Matlab Code

```
% Spatial coordinates for position across channel
x = 0:1:350;

% Maximum concentration in channel (ng/ml)
cmax = 25;

% Chemoattractant molecular weight (kilodalton)
% For this example, use EGF with molecular weight of 6 kilodalton
mw = 6;

% Convert concentration in channel into molar concentration
molarconversion = 1/(mw*10^9);

% Dissociation constant for simple (nM)
Kd = 1;

% Concentration Profiles
% Constant terms are determined by solving the equations, most easily done by assuming a
maximum concentration at the far right of the channel where x = xmax (micron):
% xmax = max(x);
% 100*(c/cmax) = A_linear*x for a linear profile
% A_linear = 100/xmax;
% 100*(c/cmax) = A*x^p for a power profile
% p = 4.2
% A_power = 100/(xmax^p)
% 100*(c/cmax) = exp(A*x)-1 for an exponential profile
% A_exp = ln(101)/xmax

% Theoretical profiles across channel
% Linear Profile
%xmax = max(x);
%A_linear = 100/xmax;
%L = (cmax/100)*(A_linear)*(molarconversion)*x;
% Power Profile
%xmax = max(x);
%p = 4.2;
%A_power = 100/(xmax^p);
%L = (cmax/100)*(A_power)*(molarconversion)*(x.^p);

% Exponential Profile
% xmax = max(x);
% A_exp = ln(101)/xmax;
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% L = (cmax/100)*(molarconversion)*(exp(A_exp*x)-1);

% Experimental profiles from Jeon paper
% Linear profile
xmax = 350;
A_linear_jeon = 100/xmax;
        L = (cmax/100)*(A_linear_jeon)*(molarconversion)*x;

% Power profile
%xmax = 400;
%p = 4.2;
%A_power_jeon = 100/(xmax^p);
%L = (cmax/100)*(A_power_jeon)*(molarconversion)*(x.^p);

% Define association constants and total receptors
    Kx0 = 5.3*10^11;
    K11 = 4.6*10^9;
    K21 = 5.3*10^9;
    K22 = 3.4*10^8;
Rt = 50000;

% Solve for unbound receptor monomers based on expression for total receptors
   a = 2*Kx0*(1 + K21*L + K21*K22*L.^2);
   b = 1 + K11*L;
   c = -Rt;
   R = (-b + (b.^2 - 4*a.*c).^.5)./(2*a);

% Define the fractional receptor term based on the system:
denominator = R.*(1+K11*L) + (2*Kx0*R.^2).*(1 + K21*L + K21*K22*L.^2);
numerator_FRA = (2*K21*K22*Kx0*R.^2).*L.^2;
numerator_FRO = K11*L.*R + (K21*Kx0*R.^2).*L + (2*K21*K22*Kx0*R.^2).*L.^2;
   FRA = numerator_FRA./denominator;
   FRO = numerator_FRO./denominator;

% Calculate DFRO/DFRA
cell_radius = 25;
channel = length(x);
for k = 1 : channel  %set incrementation to start at cell radius and progress to within a cell radius
of end of channel
if k-1<cell_radius
DFRA(k) = 0;
DFRO(k) = 0;
elseif k + cell_radius> channel
DFRA(k) = 0;
DFRO(k) = 0;
else
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DFRA(k) = FRA(k+cell_radius) - FRA(k-cell_radius);
DFRO(k) = FRO(k+cell_radius) - FRO(k-cell_radius);
end
end

% Evaluate approximate total cell migrational activity based on summed DFRA/FRA for a
specific range
FRA_ratio = DFRA./FRA;
FRO_ratio = DFRO./FRO;

% Plot Profiles
subplot(2,2,1)
plot(x,L)
title('Ligand Concentration');
ylabel('[M]');
xlabel('Position in Channel (micron)');

subplot(2,2,2)
plot(x, FRA, x, FRO)
axis([0 400 0 1]);
ylabel('Fraction');
xlabel('Position in Channel (micron)');
legend('FRA', 'FRO');

subplot(2,2,3)
plot(x, DFRA, x, DFRO)
axis([0 400 0 .5]);
ylabel('DFRA/DFRO');
xlabel('Position in Channel (micron)');
legend('DFRA', 'DFRO');

% Export data into spreadsheet for analysis
% Make row vectors into column vectors
   l = L';
FRA_export = FRA';
FRO_export = FRO';
DFRA_export = DFRA';
DFRO_export = DFRO';
FRA_ratio_export = FRA_ratio';
FRO_ratio_export = FRO_ratio';
% Export data into spreadsheet
numbers = [l, FRA_export, DFRA_export, FRA_ratio_export, FRO_export, DFRO_export,
FRO_ratio_export];
headers = {'Concentration', 'FRA', 'DFRA', 'DFRA/FRA', 'FRO', 'DFRO', 'DFRO/FRO'};
xlswrite('Data_Export.xlsx', headers, 'Workspace', 'A1');
xlswrite('Data_Export.xlsx', numbers, 'Workspace', 'A2');
```

Calculating [R]

In the aggregating receptor system, the total concentration of receptors (a fixed value) is equal to the sum of all possible receptor conformations – free receptor monomer, ligated receptor monomer, un-ligated receptor dimer, singly-ligated receptor dimer, and doubly-ligated receptor dimer: $[R_{total}]=[R]+[R\cdot L]+2[R\cdot R]+2[R\cdot R\cdot L]+2[L\cdot R\cdot R\cdot L]$. Equilibrium binding kinetics, described in the table below, were used to rearrange the equation in terms of [R] and association constants: $[R_{total}]=(1+K_{11}[EGF])[R]+2K_{x0}(1+K_{21}[EGF]+K_{21}K_{22}[EGF]^2)[R]^2$. This substitution uses microscopic equilibrium relations for K_{x2} and K_{x1} that describe them in terms of other measureable association constants where $K_{x1}=\frac{K_{x0}K_{21}}{K_{11}}$ and $K_{x2}=\frac{K_{x0}K_{21}K_{22}}{K_{11}^2}$.

Binding Equation	Association Constant	Substituting Term
$R+L \rightleftharpoons R\cdot L$	$K_{11}=\frac{[R\cdot L]}{[R][L]}$	$[R\cdot L]=K_{11}[R][L]$
$R+R \rightleftharpoons R\cdot R$	$K_{x0}=\frac{[R\cdot R]}{[R]^2}$	$[R\cdot R]=K_{x0}[R]^2$
$R\cdot R+L \rightleftharpoons R\cdot R\cdot L$	$K_{21}=\frac{[R\cdot R\cdot L]}{[R\cdot R][L]}$	
$R\cdot L+R \rightleftharpoons R\cdot R\cdot L$	$K_{x1}=\frac{[R\cdot R\cdot L]}{[R\cdot L][R]}$	$[R\cdot R\cdot L]=K_{x0}K_{21}[R]^2[L]$
$R\cdot R\cdot L+L \rightleftharpoons L\cdot R\cdot R\cdot L$	$K_{22}=\frac{[L\cdot R\cdot R\cdot L]}{[R\cdot R\cdot L][L]}$	
$R\cdot L+R\cdot L \rightleftharpoons L\cdot R\cdot R\cdot L$	$K_{x2}=\frac{[L\cdot R\cdot R\cdot L]}{[R\cdot L][R\cdot L]}$	$[L\cdot R\cdot R\cdot L]=K_{x0}K_{21}K_{22}[R]^2[L]^2$

By subtracting R_{total} from both sides, we are left with a quadratic equation for [R] where $a=2K_{x0}(1+K_{21}[EGF]+K_{21}K_{22}[EGF]^2)$, $b=(1+K_{11}[EGF])$, and $c=-[R_{total}]$. Solving this equation in terms of [R] yields the concentration of receptor monomers as a function of [EGF].

Other forms of FRA and DFRA

	Fraction of Receptors Activated	FRA described by association constants, [EGF], and [R]
1	$\frac{[R{\cdot}L]+[R{\cdot}R{\cdot}L]+2[L{\cdot}R{\cdot}R{\cdot}L]}{[R]+[R{\cdot}L]+[R{\cdot}R]+[R{\cdot}R{\cdot}L]+[L{\cdot}R{\cdot}R{\cdot}L]}$	("K"↓"11" ["EGF"]["R"]" + " "K"↓"x0" "K"↓"21" ["EG
2	$\frac{[R{\cdot}L]+2[R{\cdot}R{\cdot}L]+2[L{\cdot}R{\cdot}R{\cdot}L]}{[R]+[R{\cdot}L]+[R{\cdot}R]+[R{\cdot}R{\cdot}L]+[L{\cdot}R{\cdot}R{\cdot}L]}$	("K"↓"11" ["EGF"]["R"]" + 2" "K"↓"x0" "K"↓"21" ["E
3	$\frac{[R{\cdot}R{\cdot}L]+2[L{\cdot}R{\cdot}R{\cdot}L]}{[R]+[R{\cdot}L]+[R{\cdot}R]+[R{\cdot}R{\cdot}L]+[L{\cdot}R{\cdot}R{\cdot}L]}$	$\frac{K_{x0}K_{21}[EGF][R]^2+2K_{x0}K_{21}K_{22}[EGF]^2[R]^2}{(1+K_{11}[EGF])[R]+2K_{x0}(1+K_{21}[EGF]+K_{21}K_{22}[EGF]^2)[}$
4	$\frac{2[R{\cdot}R{\cdot}L]+2[L{\cdot}R{\cdot}R{\cdot}L]}{[R]+[R{\cdot}L]+[R{\cdot}R]+[R{\cdot}R{\cdot}L]+[L{\cdot}R{\cdot}R{\cdot}L]}$	$\frac{2K_{x0}K_{21}[EGF][R]^2+2K_{x0}K_{21}K_{22}[EGF]^2[R]^2}{(1+K_{11}[EGF])[R]+2K_{x0}(1+K_{21}[EGF]+K_{21}K_{22}[EGF]^2)[}$
5	$\frac{2[L{\cdot}R{\cdot}R{\cdot}L]}{[R]+[R{\cdot}L]+[R{\cdot}R]+[R{\cdot}R{\cdot}L]+[L{\cdot}R{\cdot}R{\cdot}L]}$	$\frac{2K_{x0}K_{21}K_{22}[EGF]^2[R]^2}{(1+K_{11}[EGF])[R]+2K_{x0}(1+K_{21}[EGF]+K_{21}K_{22}[EGF]^2)[}$

Depending on the receptor activation kinetics of the chosen model system, FRA terms can easily be modified. FRA_1 represents the same term as fractional receptor occupancy for a typical aggregating receptor system and assumes all receptor complexes are activated when ligand-bound. FRA_2 is a modified version of FRA_1; here, the factor of 2 is added to the R·R·L term to signify that *both* receptors are activated by binding a single ligand – whereas in FRA_1 only one of the receptors would be activated in the R·R·L complex. FRA_3 and FRA_4 represent systems in which only receptor dimer complexes for active signaling entities. Again, the factor of 2R·R·L in FRA_4 shows that both receptors are activated in the complex. FRA_5 is the fractional receptor activation used in this study – where only the doubly-ligated receptor dimer forms an active signaling complex.

MDA-MB-231 Migration Cannot Be Explained by Simple Cooperativity or Aggregation without Cooperativity

The simple receptor-ligand model can incorporate apparent cooperativity by varying the Hill Parameter N (Equation 2); N > 1 describes a system with positive apparent cooperativity and N < 1 is a negatively cooperative system. We analyzed and plotted FRO and DFRO for the simple model (Equation 2) with N = 0.5, 0.7, 1 (same as Wang *et al*[16]), and 2 while keeping K_d constant at 1 nM. N = 0.5 and N = 2 were chosen as representative negatively and positively cooperative Hill parameters, respectively, while N = 0.7 was calculated as the equivalent apparent Hill coefficient from the binding curves described by the EGFR aggregating system from MacDonald *et al*[20](SI Figure 1A). As can be seen, the simple system with N = 2 can describe the differential migration with little to no FRO or DFRO from 0 < x < 160 μm and significant FRO and DFRO for x > 160 μm. However, positive cooperativity is not applicable for the EGFR system as Scatchard plots show concave-up profiles indicative of negative cooperativity.[17-18,23,26] The simple systems with negative apparent cooperativity are actually worse at describing these two distinct regions as FRO and DFRO are left-shifted. Therefore, the simple model is unable to fully describe the differential migration even when taking cooperativity into account.

The aggregating receptor system can be used to calculate total FRO (Equation 3) instead of FRA as discussed previously. We calculated and plotted FRO for the aggregating receptor system with physiologically relevant dissociation constants[20] as well as theoretical constants reflective of a non-cooperative system (SI Figure 1B). FRO and DFRO are left-shifted compared to the physiologically relevant FRA and DFRA (same plot shown in Figure 3C). Because of the left shift, we would expect cells to be even more migratory for x < 160 μm and likely less migratory for x > 160 μm; therefore the DFRO aggregating system is also unable to effectively explain the different regions of chemotaxis.

Electronic Supplementary Material (ESI) for Integrative Biology