Supplementary Information

Integrin Organization: Linking Adhesion Ligand Nanopatterns with Altered Cell Responses

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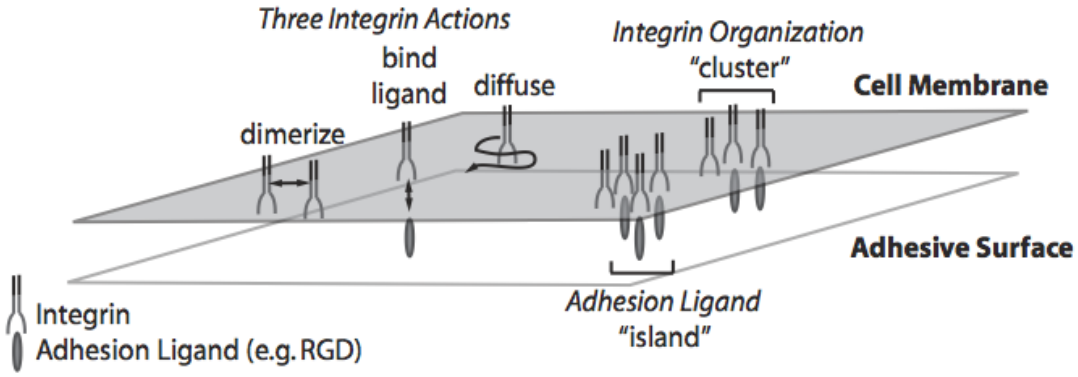
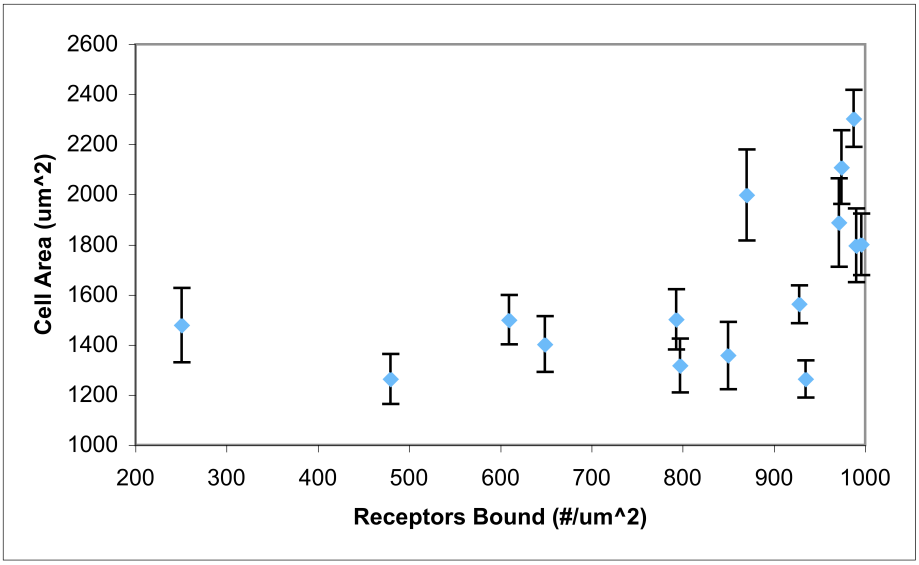


Supplementary Figure 1. Cell adhesion to an adhesive surface. Integrin receptors on the cell membrane can diffuse laterally, bind to and dissociate from adhesive ligand on the surface, and dimerize with and dissociate from other integrins. Ligand on the adhesive surface (such as RGD) may be presented singly or patterned into multivalent "islands." The ligand presentation (i.e. nanopatterning) in addition to the characteristic rate constants of the integrin actions determine the integrin organization on the cell membrane.



Supplementary Figure 2. Cell area as a function of number of receptors bound. Experimental measures of cell area (Comisar et al. 2007) are plotted as a function of predicted number of receptors bound. Data from 15 different nanopatterns (various values of F.C. and degree of substitution) are included. There is a strong correlation between cell area and receptors bound (see also Fig. 5).

Effects of altered model input parameters on integrin organization and cell responses. Altered model input parameters representing changes in the cell or adhesive surface were tested for their effect on measures of integrin organization (receptors bound, contact number, and BNDF (AUC)). Based on the correlative model described in the main text, we then predicted the effect on cell responses.

The results of these simulations are summarized in Supplementary Table 1. Complete numerical results and details can be found in (Comisar, 2007). The change to the experimental system represented by a change in particular model parameter is listed in column 1, while the corresponding change to model input is listed in column 2. Model inputs are indicated as increased or decreased based on comparison with the standard model input parameter values (see Methods). Similarly, the effect of changes to the model input parameters on receptors bound, contact number and BNDF (AUC) are compared to the values of these measures of integrin organization obtained using standard model input parameters (columns 3-5). Predictions of the effects of such changes in integrin organization on cell responses are listed in column 6.

Supplementary Table 1

Change in Cell Type/Adhesive Surface	Model Parameter	Receptors Bound	Contact Number	BNDF (AUC)	Predicted Effect
Number of integrins able to bind adhesion ligand	↑ Integrin Number ($1778/\mu m^2$)	Increased where not limited by ligand density	Same trend in response to nanopatterns, though increased at all nanopatterns	Greater difference between low and high F.C., same trend in response to nanopatterns	Higher cell spreading for lower values of F.C. and RGDs/island; increased sensitivity of cells to ligand nanopatterns; possible increase in MC3T3 osteogenic differentiation (ligand=RGD) where contact number and BNDF are both increased
	↓ Integrin Number ($355/\mu m^2$)	Significantly reduced; greatest reduction where not limited by ligand density	Not a function of nanopattern, decreased at all nanopatterns	Medium for all nanopatternam not a function of nanopattern	Lower cell spreading; greatly decreased sensitivity of cells to ligand nanopatterns
Integrins with altered ability to dimerize	↑ High Dimerization ($k_{dimer}/k_{mono}= 10^5$)	Low to medium at all nanopatterns	Less a function of nanopattern, high at all nanopatterns	Low to medium for all nanopatterns, less a function of nanopattern	Lower level of cell spreading; slightly decreased sensitivity of cells to nanopatterns
	↓ Low Dimerization ($k_{dimer}/k_{mono}= 10^1$)	Same trend, small increase where not limited by ligand density	Greater difference between low and high; same trend except decreased at high ligands/island	Same trend in response to RGD nanopatterns	Some increase in cell spreading for intermediate levels of F.C. and ligands/island. Possible increase in sensitivity of cells to RGD nanopatterns and small decrease in osteogenic differentiation of MC3T3 cells at high ligands/island and low F.C.
	↓↓ No Dimerization ($k_{dimer}=0$)	Same trend, small increase where not limited by ligand density	Greater difference between low and high; same trend except decreased at high ligands/island	Same trend in response to nanopatterns	

Different adhesion ligand	↑ Integrin-ligand affinity (k_{bind}/k_{unbind} base value x 100)	Small increase at all nanopatterns	Smaller cluster size only for high ligands/island and F.C.>0.2, same trend	Same trend, overall lower	Possible increase in pFAK Y397 for most nanopatterns
	↓ Integrin-ligand affinity (k_{bind}/k_{unbind} base value/100)	Significantly reduced at all nanopatterns —lowest levels of all simulations	Not a function of nanopattern, low at all nanopatterns	Not a function of nanopattern, low at all RGD nanopatterns	Lower cell spreading; greatly decreased sensitivity of cells to nanopatterns; possible increase in pFAK Y397 and decrease in osteogenic differentiation of MC3T3 cells