Exploring the influence of cytosolic and membrane FAK activation on YAP/TAZ nuclear translocation

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ABSTRACT Membrane binding and unbinding dynamics play a crucial role in the biological activity of several nonintegral membrane proteins, which have to be recruited to the membrane to perform their functions. By localizing to the membrane, these proteins are able to induce downstream signal amplification in their respective signaling pathways. Here, we present a 3D computational approach using reaction-diffusion equations to investigate the relation between membrane localization of focal adhesion kinase (FAK), Ras homolog family member A (RhoA), and signal amplification of the YAP/TAZ signaling pathway. Our results show that the theoretical scenarios in which FAK is membrane bound yield robust and amplified YAP/TAZ nuclear translocation signals. Moreover, we predict that the amount of YAP/TAZ nuclear translocation increases with cell spreading, confirming the experimental findings in the literature. In summary, our in silico predictions show that when the cell membrane interaction area with the underlying substrate increases, for example, through cell spreading, this leads to more encounters between membrane-bound signaling partners and downstream signal amplification. Because membrane activation is a motif common to many signaling pathways, this study has important implications for understanding the design principles of signaling networks.

INTRODUCTION

Several studies have pointed toward the influence of cell shape and spreading on cell signaling (1–7). Halder et al. (3) have shown, for example, that irrespective of substrate stiffness, Yes-associated protein (YAP) and its ortholog transcriptional coactivator with PDZ binding motif (TAZ), commonly referred to as YAP/TAZ, were mainly nuclear in spread cells on large adhesive islands. On the other hand, YAP/TAZ was mainly cytoplasmic in round cells on confined adhesive islands. By controlling organ size via the integration of mechanical stimuli (8–14), YAP and TAZ have been shown to have ubiquitous physiological roles in developmental processes, tissue homeostasis, and malignancy (8–14).

Importantly, the interactions between cells and their environment occur via transmembrane integrin molecules, of which some induce downstream YAP/TAZ translocation. Recent studies have shown that the integrin family of matrix adhesions is very heterogeneous in terms of size, subcellular distribution, and dynamic composition (15,16). Furthermore, their biological activity is dependent on their interaction with cytoplasmic proteins, which form, via binding and unbinding, the focal adhesion complex (17,18). Binding of the extracellular domains of integrins to their ECM ligands triggers conformational remodeling of their α and β subunits, which in turn leads to the presentation of binding sites to cytoplasmic proteins (16). Several theoretical and experimental studies have indicated that protein localization to a membrane, by binding to and forming membrane clusters and rafts, helps concentrate the signal to specific areas of...
the cell membrane and amplify signals from the membrane (19–24). In particular, the adsorption of a protein to a membrane surface will increase its relative probability of encountering its reaction partner, resulting in an increased reaction rate because of the higher (local) concentration (19). For example, Kholodenko et al. (19) theoretically showed that membrane localization increases the lifetime of complexes formed between signal transduction partners at the membrane, thus leading to an increased downstream signaling combined modality (cytosolic versus membrane bound, and explore the effect of spatial localization, diffusivity, and membrane (un)binding rates on downstream YAP/TAZ signaling. We seek to understand whether membrane binding has a signaling advantage and help understand the experimental observations reported in literature. We use the YAP/TAZ signaling pathway, for which we extended the well-mixed computational YAP/TAZ model of Sun et al. (27) from a one-dimensional (1D) to a 3D description, similar to Scott et al. (28). Because many signaling pathways are activated at the membrane, understanding their activation pattern and the influence on the downstream signaling thereof is of the highest importance to develop improved cell culture and organoid models as well as advanced regenerative medicine therapies.

MATERIALS AND METHODS

YAP/TAZ nuclear translocation model

We studied the relationship between cell shape, cell spreading, and YAP/TAZ nuclear translocation under five different FAK and RhoA activation modalities by using the 1D model of Sun et al. (27), which we extended to three dimensions to include spatial effects by approximating the signaling cascade as a reaction-diffusion system, similar to Scott et al. (28). We performed the implementation in Virtual Cell, a computational and simulation platform (29,30).

Briefly, in this work, we model the YAP/TAZ signaling cascade as follows (see Fig. 1). As the initial activation of the signaling cascade, we model the FAK activity and investigate the influence of cytosolic or membrane activation (see Fig. 2) (31). The first downstream effector of FAK is the small GTPase RhoA, whose active form binds to the cell membrane. Activated RhoA can in turn activate mDia and ROCK (32,33). mDia plays a role in stress fiber and filopodia formation and accelerates the elongation of actin filaments (34). ROCK acts on two downstream effectors: myosin and LIM-kinase (LIMK) (35). ROCK favors myosin activity through phosphorylation of its light chain and inhibition of myosin phosphatase (36). The activation of LIMK leads to the inactivation of cofilin, an F-actin cleaving protein (35). This actomyosin activity (i.e., contractility) and stress fiber assembly, as favored by ROCK, translates into YAP/TAZ nuclear translocation (3,8–10,37).

In silico model experiments

It has been established that the activation of RhoA occurs at the membrane with the involvement of GEFs (guanine nucleotide exchange factors), guanine nucleotide dissociation inhibitors, and GAPs (GTPase-activating proteins), whereas the inactive RhoA remains cytoplasmic (38–41). However, recent findings have shown that active and inactive forms of Rho GTPases can coexist on the membrane and can be continuously extracted from the membrane by guanine nucleotide dissociation inhibitors, with the active form being quickly recycled back to the membrane (40), which is captured in the model cases 2, 4, and 5 (see below). Interestingly, overexpression and mutations in the hypervariable regions that are involved in the localization control of the Rho GTPases have been associated with increased...
FIGURE 1 YAP/TAZ nuclear translocation signaling cascade. In our proposed model, the YAP/TAZ signaling cascade is triggered by the activation of FAK at the membrane or in the cytosol (see Fig. 2 for the different activation modalities). The net effect is the phosphorylation of YAP/TAZ under the influence of actomyosin activity, which is in turn translated into YAP/TAZ nuclear translocation. Note that we do not model the entire focal adhesion complex at the membrane but approximate this by FAK activation at the membrane.

cytosolic sequestration (42–45), which we model in cases 1 and 3 (see below).

Similarly, it has been shown that FAK is activated at the focal adhesion complex and cycles between the membrane and cytosol with focal adhesion assembly and disassembly (46,47), which we model in case 5 (see below). On the one hand, point mutations in the FAT domain of FAK can inhibit FAK recruitment to focal adhesions by abrogating FAK-paxillin interaction (48,49), resulting in exclusively cytoplasmic FAK. In addition, other authors have reported that active FAK can be found in the cytoplasm (which we model in cases 1 and 2; see below), and even translocate from the cytoplasm to the nucleus (46,50,51). On the other hand, Emelia and Tuma-barello (52) have shown that depletion of FIP200 and loss of Atg5 (two autophagosome regulators) lead to retention of active FAK at focal adhesions, which we model in cases 3 and 4 (see below).

Importantly, the particular localization of FAK or RhoA has been shown to influence downstream YAP/TAZ signaling. For example, Valon et al. (53) showed that plasma membrane localization of an engineered optogenic RhoA activator (optoGEF-RhoA) leads to YAP nuclear localization, whereas mitochondrial localization is associated with a decrease in nuclear YAP. Similarly, lovastatin, a small molecule that prevents membrane localization of RhoA, was found to inhibit the nuclear localization of YAP (54,55). Moreover, alternative splicing of FAK, shown to result in reduced focal adhesion formation, increased the cytoplasmic localization of FAK and reduced nuclear YAP (56,57).

In summary, to adequately compare the effect of cytoplasmic and membrane localization of RhoA and FAK on the YAP/TAZ nuclear translocation response and based on the previous observations, each of the following five model cases was chosen such that we could progressively build from an all-cytosolic localization of RhoA and FAK to different scenarios of membrane localization (see Fig. 2).

1. Case 1: FAK and RhoA are cytosolic and get activated in the cytoplasm. They both freely diffuse within the cytoplasm.
2. Case 2: FAK is cytosolic and RhoA is membrane bound (part of the focal adhesion complex); FAK is activated in a small region (0.5 μm in height) close to the membrane at the base of the cell.
3. Case 3: FAK is membrane bound and does not diffuse within the membrane (part of the focal adhesion complex). RhoA is cytosolic and freely diffuses within the cytoplasm.
4. Case 4: FAK and RhoA are both membrane bound (part of the focal adhesion complex), and RhoA can bind and unbind from the focal adhesion complex. Additionally, FAK does not diffuse within the focal adhesion complex, but RhoA can diffuse within the membrane and in the cytoplasm.
5. Case 5: FAK and RhoA are both membrane bound (part of the focal adhesion complex); they can both bind and unbind from the focal adhesion complex. FAK does not diffuse within the focal adhesion complex, whereas RhoA can diffuse within the membrane. Both FAK and RhoA diffuse within the cytoplasm.

The 0.5 μm band in case 2 is used to represent a very small zone of activation of FAK close to the membrane, which models the focal adhesion region without explicitly segregating FAK from the cytosol. We chose 0.5 μm because it was the smallest distance that could be achieved at the mesh size we used while keeping the same initial FAK number of molecules across cases.

Model equations

In the computational model, all proteins have an active and inactive form; G-actin represents the inactive form of F-actin. The active form represents the phosphorylated state, except for cofilin, which gets activated upon dephosphorylation (35). The dynamics of the active and inactive forms of the signaling molecules are described in general terms in Eqs. 1 and 2.

Active forms (general equation) are as follows:

$$\frac{dS}{dt} = Q_v \times S_i - d_S \times S + D_v \times \nabla^2 S,$$  \hspace{1cm} (1)

The active protein is denoted with $S$, and the inactive is denoted $S_i$. $Q_v$ represents the activation rate of the inactive form, and $d_S$ represents the degradation rate of the active form (in both Eqs. 1 and 2). $D_v$ represents the diffusion coefficient.

Inactive forms (general equation) are as follows:

$$\frac{dS_i}{dt} = d_S \times S - Q_v \times S_i + D_S \times \nabla^2 S_i.$$  \hspace{1cm} (2)

The equations for the dynamics of specific proteins presented in Eqs. 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15 focus on the active forms and are adapted from Sun et al. (27). Because we focus on YAP/TAZ signaling resulting from FAK activation only, we removed the baseline activation of RhoA, Myosin, LIMK, and F-actin by other pathways, i.e., independent from the FAK signaling cascade, as well as the LATS-related terms (LATS\_n and LATS\_p) and the constitutive baseline activation of YAP/TAZ ($K_{const}$). All the parameter values are found in Table 1, and the equations are further detailed below.

The model equations Eqs. 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, and 15 describe the standard scenario (case 5). In this scenario, both FAK and RhoA can cycle between their membrane-bound forms and their cytosolic freely diffusing forms. Inspired by Spill et al. (67) and Holmes et al. (68), we distinguish between membrane-bound inactive FAK ($FAK_m$) and RhoA ($RhoA_m$), membrane-bound active FAK ($FAK$) and RhoA ($RhoA$), and cytosolic inactive FAK ($FAK_s$) and RhoA ($RhoA_s$). Importantly, although we acknowledge the complex and dynamic composition of the focal adhesion complex, we approximate it here by a FAK activation at the membrane.
Inactive cytosolic FAK (FAKci) diffuses in the cytosol with a diffusion coefficient $D_{FAKci}$ (Eq. 3). It then binds to the membrane in a reversible manner at respective binding and unbinding rates $K_{fon}$ and $K_{foff}$ (see Boundary conditions). The membrane-bound form of inactive FAK (FAKmi) results from the inactivation of the active bound form of FAK (FAK). The YAP/TAZ signaling cascade is initiated by setting a predetermined initial amount of active FAK, which then decays with time (Eq. 5).

$$\frac{\partial FAK_{ci}}{\partial t} = D_{FAKci} \times \nabla^2 FAK_{ci}. \quad (3)$$

$$\frac{\partial FAK_{mi}}{\partial t} = K_{df} \times FAK + D_{FAKmi} \times \nabla^2 FAK_{mi}. \quad (4)$$

$$\frac{\partial FAK}{\partial t} = -K_{df} \times FAK + D_{FAK} \times \nabla^2 FAK. \quad (5)$$

Just like the inactive cytosolic FAK, the inactive cytosolic RhoA (RhoAci) only diffuses in the cytosol with diffusion coefficient $D_{RhoAci}$ (Eq. 6).

$$\frac{\partial RhoA_{ci}}{\partial t} = D_{RhoAci} \times \nabla^2 RhoA_{ci}. \quad (6)$$

Inactive cytosolic RhoA binds to the membrane with a rate $K_{ra}$ (see Boundary conditions). The inactive membrane-bound RhoA unbinds at a rate $K_{ar}$, diffuses on the plasma membrane with a rate $D_{RhoAmi}$, gets activated by FAK at a rate $K_{fr}$, and degrades at a rate $dK_{d}$. (Eq. 8).

$$\frac{\partial RhoA}{\partial t} = K_{fr} FAK_{m} RhoA_{mi} - K_{ar} RhoA + D_{RhoA} \nabla^2 RhoA. \quad (8)$$

In Eq. 8, $K_{d}$ is the degradation rate of ROCK, and $D_{ROCK}$ is the diffusion coefficient of ROCK.

$$\frac{\partial ROCK}{\partial t} = -K_{drock} \times ROCK + D_{ROCK} \times \nabla^2 ROCK. \quad (9)$$

In Eq. 9, $K_{d}$ is the degradation rate of ROCK, and $D_{ROCK}$ is the diffusion coefficient of ROCK. (mDia): $dK_{d}$ is the degradation rate of ROCK, and $D_{ROCK}$ is the diffusion coefficient of ROCK.

$$\frac{\partial mDia}{\partial t} = -K_{dm} \times mDia + D_{m} \times \nabla^2 mDia. \quad (10)$$

In Eq. 10, $K_{dm}$ is the degradation rate of ROCK, and $D_{ROCK}$ is the diffusion coefficient of ROCK. (mDia): $dK_{d}$ is the degradation rate of ROCK, and $D_{ROCK}$ is the diffusion coefficient of ROCK.
| Parameter | Definition | Value | References |
|-----------|------------|-------|------------|
| $K_{my}$ | FAK-dependent RhoA phosphorylation | $9 \text{ s}^{-1}$ | (27) |
| $mDiag$ | mDia threshold | 0.13 µM | (27) |
| $K_{d}$ | RhoA dephosphorylation | 0.625 s$^{-1}$ | (58) |
| $ROCK_p$ | ROCK threshold | 0.26 µM | (27) |
| $LD$ | ligand density | 2 | (29) |
| $C$ | ligand density × Emol when the FAK activation is $K_{my}/2$ | 45 µM | (27) |
| $K_{df}$ | FAK dephosphorylation rate | 0.035 s$^{-1}$ | (59) |
| $K_{df}$ | FAK activation rate due to integrin activation | 0.3795 s$^{-1}$ | (59) |
| $K_{dROCK}$ | RhoA-dependent ROCK activation | 2.2 s$^{-1}$ µM$^{-1}$ | (60,61) |
| $K_{ROCK}$ | ROCK-dependent LIMK activation rate | 0.8 s$^{-1}$ | (27) |
| $K_{act}$ | LIMK degradation rate | 2 s$^{-1}$ | (27) |
| $K_{act}$ | ROCK-dependent LIMK activation amplification | 200 µM$^{-1}$ | (27) |
| $K_{df}$ | ROCK-dependent LIMK activation | 1 s$^{-1}$ | (27) |
| $K_{ddf}$ | ROCK-dependent mDia activation | 1 s$^{-1}$ µM$^{-1}$ | (27) |
| $K_{dfy}$ | myosin activation rate | 0.015 s$^{-1}$ | adapted from (27) |
| $K_{dfy}$ | myosin degradation rate | 0.067 s$^{-1}$ | (62) |
| $\epsilon$ | ROCK-dependent myosin activation | 40 µM$^{-1}$ | (27) |
| $E$ | stiffness of substratum | $10^6$ kPa | (63,64) |
| $K_{turnover}$ | cofillin dephosphorylation rate | 0.04 s$^{-1}$ | (65) |
| $K_i$ | LIMK-dependent cofillin phosphorylation rate | 0.7 µM$^{-2}$ s$^{-1}$ | (41) |
| $\alpha$ | mDia-dependent F-actin activation amplification | 40 µM$^{-1}$ | (27) |
| $K_{dfp}$ | F-actin depolymerization rate | 3.5 s$^{-1}$ | (66) |
| $K_{dfp}$ | cofillin-dependent F-actin severing rate | 8 s$^{-1}$ µM$^{-1}$ | (27) |
| $K_f$ | F-actin polymerization rate | 0.4 s$^{-1}$ | (27) |
| $K_{mF}$ | YAP/TAZ nuclear import rate | 0.4 s$^{-1}$ | fitted |
| $K_f$ | cytoplasmic YAP/TAZ phosphorylation rate | 20 µM$^{-1}$ s$^{-1}$ | (27) |
| $K_{dfy}$ | cytoplasmic YAP/TAZ dephosphorylation rate | 0.1 µM$^{-1}$ s$^{-1}$ | (27) |
| $K_{Fm}$ | FAK membrane binding rate | 0.029 s$^{-1}$ | (18) |
| $K_{Ff}$ | FAK membrane unbinding rate | 0.017 µm$^{-1}$s$^{-1}$ | (18) |
| $K_m$ | RhoA membrane binding rate | 20 s$^{-1}$ | estimated |
| $K_m$ | RhoA membrane unbinding rate | 0.5 µm$^{-1}$s$^{-1}$ | estimated |
| $D_{FAKc}$ | diffusion coefficient of inactive cytosolic FAK | 15.96 µm$^2$s$^{-1}$ | calculated |
| $D_{ROKc}$ | diffusion coefficient of inactive cytosolic RhoA | 28.03 µm$^2$s$^{-1}$ | calculated |
| $D_{ROK}$ | diffusion coefficient of active RhoA | 0.06 µm$^2$s$^{-1}$ | calculated |
| $D_{ROCK}$ | diffusion coefficient of ROCK | 11.39 µm$^2$s$^{-1}$ | calculated |
| $D_m$ | diffusion coefficient of mDia | 15.16 µm$^2$s$^{-1}$ | calculated |
| $D_{dfy}$ | diffusion coefficient of myosin | 9.76 µm$^2$s$^{-1}$ | calculated |
| $D_{LIMKi}$ | diffusion coefficient of LIMK | 18.82 µm$^2$s$^{-1}$ | calculated |
| $D_{m}$ | diffusion coefficient of cofillin | 29.44 µm$^2$s$^{-1}$ | calculated |
| $D_{m}$ | diffusion coefficient of cofillin | 0.001 µm$^2$s$^{-1}$ | calculated |
| $D_{cactin}$ | diffusion coefficient of G-actin | 22.58 µm$^2$s$^{-1}$ | calculated |
| $D_{YAPTAZ}$ | diffusion coefficient of cytoplasmic active YAP/TAZ | 20.71 µm$^2$s$^{-1}$ | calculated |

ROCK acts on two downstream effectors; myosin and LIMK. ROCK favors myosin activity through phosphorylation of its light chain and inhibition of myosin phosphatase (35).

$\text{Myo}$:

$$\frac{\partial \text{Myo}}{\partial t} = K_{my} \times \epsilon \times T_{ROCK} \times \text{Myo} - K_{dfy} \times \text{Myo} + D_{my} \times \nabla^2 \text{Myo}.$$  

(11)

The activation of myosin is represented by $K_{my}$, the degradation rate is $K_{dfy}$, and its diffusion coefficient is $D_{my}$ (Eq. 11). Here, $\epsilon$ is the active myosin amplification rate by active ROCK.

$LIMK$:

$$\frac{\partial \text{LIMKi}}{\partial t} = K_{fr} \times \tau \times T_{ROCK} \times \text{LIMKi} - K_{r} \times \text{LIMKi} + D_{LIMKi} \times \nabla^2 \text{LIMKi}.$$  

(12)
The activation rate of LIMK is $K_{\text{on}}$, and $K_{\text{off}}$ is the degradation rate and $D_{\text{LIMK}}$ the diffusion coefficient (Eq. 12). $T_{\text{ROCK}}$ is the corresponding threshold function for ROCK (see below). The activation of LIMK leads to the inactivation of coflin, an F-actin cleaving protein.

Cofilin:

$$\frac{\partial \text{Cofilin}}{\partial t} = K_{\text{turnover}} \times \text{Cofilin}_i - K_{d} \times \text{LIMK}^2 \times \text{Cofilin} + D_e \times \nabla^2 \text{Cofilin}.$$  

(13)

In Eq. 13, $K_{\text{turnover}}$ is the coflin activation rate, $K_d$ is the coflin deactivation rate, and $D_e$ is the diffusion coefficient of cofilin. mDia and cofilin are involved in the assembly and disassembly of filamentous actin from inactive to globular G-actin subunits, respectively.

Cytosolic F-actin:

$$\frac{\partial \text{Fcyto}}{\partial t} = K_f \times \alpha \times T_{\text{mDia}} \times \text{mDia} \times \text{Gactin} - K_{\text{dep}} \times \text{Fcyto} - K_{d}\text{cy} \times \text{Cofilin} \times \text{Fcyto} + D_{\text{Fcyto}} \times \nabla^2 \text{Fcyto}.$$  

(14)

In Eq. 14, $K_f$ is the assembly rate, $K_{\text{dep}}$ the depolymerization rate, $K_{\text{d}\text{cy}}$ the disassembly rate, and $D_{\text{Fcyto}}$ the diffusion coefficient of F-actin. $T_{\text{mDia}}$ is the corresponding threshold function for mDia (see below). G-actin, considered as the inactive form of F-actin, diffuses with diffusion coefficient 22.58 $\mu$m$^2$ s$^{-1}$, estimated from the Stokes-Einstein relation (69). Here, we assign an arbitrary low diffusion coefficient (i.e., 0.001 $\mu$m$^2$ s$^{-1}$) to F-actin because it is a filamentous protein made up of G-actin subunits.

Cofilin and Myosin influence the activation of the inactive cytosolic YAP/TAZ. The active cytosolic YAP/TAZ subsequently translocates into the nucleus. As such, we distinguish between inactive cytoplasmic YAP/TAZ (\emph{YAPTAZ}$_c$), active cytoplasmic YAP/TAZ (\emph{YAPTAZ}$_{ci}$), and nuclear YAP/TAZ (\emph{YAPTAZ}$_n$) which refers to the amount of \emph{YAPTAZ} which is shuttled in the nucleus (see \emph{Boundary conditions}; Eqs. 15 and 24).

\emph{YAPTAZ}$_c$ (active cytoplasmic YAP/TAZ):

$$\frac{\partial \text{YAPTAZ}_c}{\partial t} = K_f \times \text{Fcyto} \times \text{Myo} \times \text{YAPTAZ}_{ci} - K_{d}\text{cy} \times \text{YAPTAZ}_c + D_{\text{YAPTAZ}_c} \times \nabla^2 \text{YAPTAZ}_c.$$  

(15)

In Eq. 15, inactive YAP/TAZ (\emph{YAPTAZ}$_{ci}$) gets activated by phosphorylation at a rate $K_f$, and gets deactivated by dephosphorylation at a rate $K_{d}\text{cy}$. $D_{\text{YAPTAZ}_c}$ is the diffusion coefficient of active cytosolic YAP/TAZ. The notion that ROCK and mDia concentrations have to exceed a threshold value to trigger LIMK and G-actin activation, respectively, is approximated by a threshold T function (Eqs. 16 and 17), similar to (27). The linear region of the T function corresponds to a scenario in which the ROCK or mDia concentration value is above ROCK$_B$ or mDia$_B$.

$$T_{\text{ROCK}} = \begin{cases} 0 & \text{when } \text{ROCK} \leq \text{ROCK}_B \\ \text{ROCK} - \text{ROCK}_B & \text{when } \text{ROCK} > \text{ROCK}_B \end{cases}.$$  

(16)

$$T_{\text{mDia}} = \begin{cases} 0 & \text{when } \text{mDia} \leq \text{mDia}_B \\ \text{mDia} - \text{mDia}_B & \text{when } \text{mDia} > \text{mDia}_B \end{cases}.$$  

(17)

In the results, we calculate the YAP/TAZ nuclear fraction (\emph{YNFN}) as follows:

$$Y\text{TNF} = \frac{\text{YAPTAZ}_n(\text{number of molecules})}{\text{YAPTAZ}_c + \text{YAPTAZ}_{ci} + \text{YAPTAZ}_n(\text{number of molecules})}.$$  

Boundary conditions

The boundary conditions represent mathematically what happens at the boundaries of the specified domain, i.e., whether components can enter or leave the system or move from one domain (e.g., the cytoplasm) to another (e.g., the nucleus).

\emph{Boundary condition at the plasma membrane for FAK, RhoA, ROCK, and mDia}

The boundary condition for FAK, case 5 (see Eq. 18 below), at the plasma membrane is such that the (un)binding events are in balance with the diffusive flux:

$$-D_{\text{FAK}_{ci}} \times e_n \times \nabla_i \text{FAK}_{ci} = (K_{F_{on}} \times \text{FAK}_{ci} - N \times K_{F_{off}} \times \text{FAK}_{mi}).$$  

(18)

For RhoA in cases 2, 4, and 5, we have a boundary condition as follows:

$$-D_{\text{RhoA}_{ci}} \times e_n \times \nabla_i \text{RhoA}_{ci} = (K_{on} \times \text{RhoA}_{ci} - N \times K_{off} \times \text{RhoA}_{mi}),$$  

(19)

where $V$ is the cytosol domain, $e_n$ the unit outward normal vector at the membrane, and the terms $e_n \times \nabla_i \text{FAK}_{ci}$ and $e_n \times \nabla_i \text{RhoA}_{ci}$ the projection of the gradient of FAK$_{ci}$ and RhoA$_{ci}$ on the unit normal vector on the surface, similar to Spill et al. (67).

In case 3, RhoA$_{ci}$ has the following boundary condition because it gets activated by interacting with active FAK at the membrane:

$$-D_{\text{RhoA}_{ci}} \times e_n \times \nabla_i \text{RhoA}_{ci} = N \times K_{f\rho} \times \text{FAK}^2 \times \text{RhoA}_{ci}.$$  

(20)

ROCK and mDia have the following boundary conditions in cases 2, 4, and 5 because they get activated by interacting with active RhoA at the membrane:

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TABLE 2  Initial concentrations used in the YAP/TAZ nuclear translocation model

| Species | Inactive | Active |
|---------|----------|--------|
| FAK<sup>a</sup> | 1,260,018.86 molecules (0.75–2.0 μM) | – |
| FAK<sub>off</sub> | 0 μM | – |
| FAK<sup>b</sup> | – | 58,104.35 molecules (0.035–0.25 μM) |
| Cofilin | 1 μM | 0 μM |
| RhoA<sub>m</sub> | 1 μM | – |
| RhoA<sub>off</sub> | 0 μM | – |
| RhoA | – | 0 μM |
| LIMK | 1 μM | 0 μM |
| Actin | 0 μM (G-actin) | 1 μM (F-actin) |
| YAP/TAZ<sub>i</sub> | 1 μM | – |
| YAP/TAZ<sub>c</sub> | – | 0 μM |
| YAP/TAZ<sub>n</sub> | – | 0 μM |
| FAK | 3.5 μM | 0 μM |
| mDia | 1 μM | 0 μM |
| ROCK | 1 μM | 0 μM |
| myosin | 1 μM | 0 μM |

Note that G-actin is the inactive form of F-actin. Note that when Virtual Cell determines the number of molecules, based on the domain volume and specified concentration, it does not round off to the nearest whole number.

<sup>a</sup>The initial number of molecules of inactive (cytosolic) form correspond to 0.75 μM in cases 1, 2, and 5 and 2.0 μM in cases 3 and 4 for a standard cell of base radius 16 μm.

<sup>b</sup>The number of molecules of the active form correspond to a concentration of 0.035 μM for case 1, 0.36 μM for case 2, and 0.25 μM for cases 3–5. The values are within similar ranges for total FAK or signaling protein initial FAK concentration (27,28,77–79).

Initial conditions and diffusion coefficients

The diffusion coefficients are as shown in Table 1. Note that the active and inactive forms have the same diffusion coefficients except otherwise mentioned. We assume that FAK diffuses in the cytosol, but not on the plasma membrane. The values for the diffusion coefficients were obtained from the Stokes-Einstein equation (69) (details in Supporting materials and methods), and we assigned the same calculated value of the diffusion coefficient to the inactive and active forms for all species that we considered unbound to the plasma membrane when activated. For RhoA, which we considered bound to the membrane in cases 3–5, and based on Marée et al. (2), we modeled diffusion such that the cytosolic inactive RhoA diffused 500 times faster than its active membrane-bound counterpart (active RhoA). This difference between membrane-bound and cytosolic diffusivities is based on the observations of Ueda et al. (70) on G-protein kinase in chemotactic signaling and the estimations of Postma et al. (71), who showed that membrane-bound proteins were able to diffuse much faster in the cytosol than when bound to the plasma membrane. In addition, by using expectation maximization on in vivo membrane-bound Rho GTPase data from single-molecule tracking photoactivated microscopy, Koo et al. (72) identified various Rho GTPases diffusion coefficients ranging from 0.0007 μm<sup>2</sup> s<sup>−1</sup> to around 0.7 μm<sup>2</sup> s<sup>−1</sup>, which supports the value used in this study (0.06 μm<sup>2</sup> s<sup>−1</sup>). The discrepancy between membrane-bound and cytosolic diffusivities could be explained by several factors inherent to the plasma membrane dynamics, including diffusion attenuation structures such as the cytoskeleton meshwork, the existence of lipid microdomains and rafts, and protein-protein interactions (73–75). Note should be taken that because F-actin is a filamentous protein, it was assigned a very low diffusion coefficient of 0.001 μm<sup>2</sup> s<sup>−1</sup>. The diffusion coefficient of YAP/TAZ was determined from the average of the molecular weight of YAP and TAZ.

The initial concentrations are as shown in Table 2, with all species in the YAP/TAZ signaling pathway initially inactive, except for FAK. The FAK input is such that the initial number of active and inactive FAK molecules are kept constant across all experiments. This initial amount of active FAK is set to match the amount of active FAK in (28), and we fitted the YAP/TAZ nuclear import rate to obtain a YAP/TAZ nuclear fraction (YTNF) of 80% for case 5 of the FAK activation mode (see In silico model experiments).
similar to what is reported in literature (76) for a stiffness value classified as “high” (23 kPa) for a similar cell volume as the standard cell in this study (base radius 16 μm). To find the best fitting import rate, we varied this parameter while keeping all other parameter values at their standard values as described in Tables 1 and 2.

In this study, we contrast two types of initial FAK activation. In a first approach, we trigger the YAP/TAZ signaling dynamics by defining a predetermined initial amount of active (and inactive) FAK (see Table 2). In the second approach, inactive FAK is activated at a particular activation rate for 100 s, including the effect of substrate stiffness (see Eq. S2). As such, initially, there is no active FAK, and the total amount of inactive FAK is set equal to the total amount of FAK (active plus inactive) of the first modality.

Geometry

We obtained a previously defined theoretical realistic cell shape (80) in Virtual Cell, in which we implemented our in silico experiments. This geometry was also used by Scott et al. (28), which will enable easier comparison. Although this is an approximate geometry, its analytical expression is shown to best approximate a fibroblast with a discoid base as presented in Schneider and Haugh (81). Experiments were performed for all five cases for spreading cells with four base cell radii of 14, 16, 18, and 20 μm. The standard cell has a base radius of 16 μm.

In all instances, the cell volume was kept constant at 2925 μm$^3$, the distance from the nucleus to the cell membrane at the base was 4 μm, and the nuclear volume was 125 μm$^3$. The nucleus was centered with respect to the base of the cell (Fig. S1).

Numerical implementation

In Virtual Cell, we used the fully implicit finite volume regular grid solver with a variable time step (range 0–0.1 s) to find numerical solutions to the partial differential equations describing the signaling cascade. This solver uses the finite volume method to represent partial differential equations as algebraic discretization equations, which exactly preserves conservation laws and employs a Sundials stiff solver CVODE for time stepping (method of lines). The values are calculated at discrete places on a meshed geometry. We used a 3D grid with 80 × 33 × 25 elements (1 element = 0.58 × 0.69 × 0.67 μm) with absolute tolerance 10$^{-4}$ and relative tolerance of 10$^{-7}$. A Virtual Cell mesh is a set of discrete elements (here 3D) defining the spatial domain on which the mathematical operations of spatial solvers occur. Virtual Cell meshes are regular grids created by dividing space (geometry size) in each dimension for forming a lattice of cells (mesh size). We performed a mesh convergence analysis and selected this mesh size as a good tradeoff between simulation time and accuracy (results not shown). We used the high-performance computing infrastructure of the Center for Cell Analysis and Modeling of the University of Connecticut Health campus at Farmington, CT, to remotely run our simulations in Virtual Cell. All the simulation code is available in the Virtual Cell repository, see Supporting materials and methods for details.

RESULTS

Membrane localization of FAK is important for YAP/TAZ nuclear translocation

Using the computational model developed above, we explored how different FAK and RhoA activation scenarios, as well as cell shape and spreading, influence YAP/TAZ nuclear translocation. Experimental and computational evidence has demonstrated that YAP/TAZ nuclear translocation is influenced by cell shape (1–7, 27); here, we asked how nuclear translocation is transiently influenced by the activation mode, i.e., cytosolic (cases 1 and 2) versus membrane-bound activation (cases 3–5).

Fig. 3 shows an overview of the spatiotemporal dynamics of FAK, RhoA, and YAP/TAZ for the different activation modes, in which the total initial amount of FAK is kept constant for comparison. Downstream of FAK, RhoA gets activated, which in turn activates downstream signaling components, resulting ultimately in YAP/TAZ nuclear translocation (see also Fig. 1). Interestingly, for cases 1 and 2, in which FAK is initially activated in the cytosol (case 1) or in a region close to the membrane (case 2), there is no YAP/TAZ nuclear translocation (0 μM) in comparison to cases 3–5, in which FAK is activated on the plasma membrane, resulting in 16.44 μM nuclear YAP/TAZ concentration for case 3 and 11.23 μM for cases 4 and 5 at 100 s. Because of differences in dimensionality of the membrane (2D) and cytoplasm (3D), the same initial amount of FAK molecules translates into a higher initial FAK concentration at the membrane and thus higher downstream activation rates and ultimately YAP/TAZ nuclear concentrations for cases 3–5. Indeed, for the standard cell size and initial number of FAK molecules, when RhoA and FAK do not colocalize at the membrane through (un)binding, it is impossible to trigger strong enough signals downstream to exceed the ROCK and mDia thresholds (see Eqs. 16 and 17). However, higher amounts of initial active FAK do result in YAP/TAZ nuclear translocation for cases 1 and 2 (see Fig. S6). Another unexpected observation relates to the fact that in cases 4 and 5, the active RhoA concentration continues to increase over time, despite active FAK decreasing over time, whereas in case 3 the active RhoA returns to zero together with FAK (even though the activation and deactivation rate of RhoA is the same for all cases). Indeed, because of the particular membrane binding and unbinding dynamics of RhoA in cases 4 and 5, including cytosolic inactive, membrane-bound inactive, and membrane-bound active species, the amount of inactive RhoA available for activation at the membrane changes. Moreover, in cases 4 and 5, both RhoA and FAK are membrane bound and thus in close proximity. As such, the active RhoA gradually rises in cases 4 and 5, whereas the inactive RhoA is almost immediately activated in case 3 (see also Fig. 5 B below). In summary, these results are in line with the known relevance of membrane protein localization for downstream signal amplification.

YAP/TAZ nuclear translocation increases with cell spreading when RhoA is membrane bound

Because YAP/TAZ has been shown to be principally nuclear in spread cells compared to confined cells where YAP/TAZ is located in the cytoplasm (3, 8), we next explored the effect of cell spreading under the various FAK activation modes as described earlier (cases 3–5). We focus here on cases 3–5, as
cases 1 and 2 did not show YAP/TAZ nuclear translocation at standard settings. Fig. 4 shows that the YAP/TAZ fraction increases with time for all activation modes, although for case 3 the nuclear fraction starts to increase earlier and reaches the steady-state concentration faster than for cases 4 and 5. For cases 4 and 5, there was also an increase in steady-state YAP/TAZ nuclear fraction (YTNF) with increased cell spreading (Fig. 4). For case 3, an earlier signaling response is observed than for cases 4 and 5, in which the peak concentrations of active RhoA, F-actin, and active myosin are reached at an earlier time point in contrast to cases 4 and 5. The earlier signaling response of case 3 resulted in smaller peak concentrations of active RhoA but similar peak concentrations of F-actin and higher peak concentrations of active myosin with respect to cases 4 and 5 (Fig. 5, B–D). Similar to what was observed above for the YTNF, there was a slight decrease in RhoA and F-actin peak concentration with increased cell spreading for case 3 (Tables S2 and S3). Interestingly, whereas in Fig. 4 an increase in YTNF with increased cell spreading was observed for cases 4 and 5, Fig. 5 shows a reduction in peak RhoA concentration with increased cell spreading (see Supporting materials and methods for quantification; Table S2). Similarly, F-actin showed a decrease in concentration with increased cell spreading for cases 4 and 5, whereas myosin showed an increase in concentration with increased cell spreading (Table S4). These results point toward a diversity of signaling concentrations and timing in response to cell spreading and different activation modes.
The above findings illustrate that when both RhoA and FAK are membrane bound, this will lead to downstream signal amplification and increased YAP/TAZ nuclear translocation with increased cell spreading. More specifically, with increased cell spreading, for all cases the local amount of FAK available per unit area to interact with RhoA decreases as the number of active FAK molecules is kept constant across cell sizes and cases. As such, for case 3 in which increased cell spreading does not change the RhoA concentration, this decrease in local FAK concentration results in less RhoA activation and a lower YAP/TAZ fraction with increased cell spreading. However, for cases 4 and 5, this effect can be compensated by the increased surface area. Indeed, the ability for FAK and RhoA to react after RhoA binding to the membrane (thereby increasing its local concentration) is larger because of the increased surface area, resulting in more active RhoA and downstream activation.

**Diffusion coefficients of RhoA and FAK and their binding dynamics influence YAP/TAZ nuclear translocation**

Diffusion coefficients and binding kinetics are important factors that can affect biological signaling (82,83). Here, we wanted to explore the effect of FAK and RhoA diffusion coefficients on YAP/TAZ nuclear translocation for different cell spreading and activation cases (Figs. 6 and S3).

For case 3, the YTNF decreases with increased diffusion of membrane-bound active FAK (0.75 at 0.003192 μm² s⁻¹ vs. 0.72 at 0.3192 μm² s⁻¹ for the standard cell size), whereas for cases 4 and 5, the YTNF increases with increased diffusion of membrane-bound active FAK. Interestingly, the influence of the diffusion coefficient of active membrane-bound FAK becomes less important with increased cell spreading (Fig. 6 A). This finding is aligned with the above explanation, in which the increased cell spreading results in a lower local FAK concentration but an increased local RhoA concentration (after membrane binding) (for cases 4 and 5). The increased diffusion further increases the surface area in which RhoA and FAK can react, although increased cell spreading counteracts this effect due to the increased diffusion distances. The diffusion coefficients of the inactive forms of FAK have no effect on the YAP/TAZ nuclear response.

For case 3, the YTNF increased with increased diffusion coefficient of the inactive form of RhoA for all cell spreading states up to a saturation level (Fig. 6 C), whereas the YTNF was not influenced by the diffusion coefficient of active cytosolic RhoA (Fig. 6 D). For cases 4 and 5, the diffusion coefficient of the inactive cytosolic form of RhoA did not influence the YAP/TAZ fraction (Fig. 6 C), whereas the YAP/TAZ fraction slightly increased with increased diffusion coefficient of the inactive membrane-bound form of RhoA, although this effect was reduced with increased cell spreading (Fig. S3). Interestingly, the YAP/TAZ fraction decreased with increased diffusion coefficient of the active membrane-bound form of RhoA, although this effect was also reduced with increased cell spreading (Fig. 6 D). These results are again aligned with the above explanations that cell spreading results in an increased surface area for RhoA binding and RhoA activation.

Increasing or decreasing the binding rate of inactive cytosolic FAK in case 5 did not affect the YTNF (Fig. S3). Contrarily, an increase in the binding rate of inactive RhoA resulted in an increase in YTNF, whereas the opposite occurred for the unbinding rate of membrane-bound RhoA in cases 4 and 5 (Fig. 6 E and F). Also here, the influence of the binding rates reduced with increased cell spreading.

Together, these results show that the diffusion coefficients and binding dynamics of RhoA are more important for YAP/TAZ nuclear translocation than those of FAK under these model settings and that cell spreading has a dampening effect.

**FAK unbinding and binding rates influence YAP/TAZ nuclear translocation under sustained activation**

In the above simulations, no differences were observed between cases 4 and 5 because the simulations were initialized with a particular amount of active FAK, bypassing the (un)binding process that distinguishes these two cases (see Fig. 2). To explore the influence of FAK (un)binding, we
modified the initial condition so that membrane-bound inactive FAK is activated at an activation rate $K_{sf}$ for 100 s (see Eq. S2). Note that because of the influence of cell spreading and FAK (un)binding, this results in different amounts of active FAK and consequently downstream signaling (Figs. 7, S4, and S5). With these settings, all YAP/TAZ translocates to the nucleus for case 4, whereas for case 5, the amount of YAP/TAZ translocation varies between 0.12 and 0.54, pointing toward a high sensitivity with respect to degree of cell spreading (Fig. 7 A). The sustained activation signal (compare with and without $K_{sf}$ in Fig. 7) also results in sustained downstream signaling. It is interesting to note that the signaling starts earlier for case 4 because all the inactive FAK is already at the membrane (and can immediately get activated), whereas for case 5 the inactive cytosolic FAK first needs to bind to the membrane.

As expected, the steady-state YTNF increases with increasing FAK binding rate (Fig. 8 A) and decreases with increased FAK unbinding rate, although this effect is (partially) countered with increased cell spreading (Fig. 8 B). Similar observations can be made for RhoA (Fig. 8, C and D), although case 4 is less sensitive to the RhoA (un)binding rates than case 5. Indeed, in case 4 RhoA is activated earlier (see Fig. 7 B) because the inactive FAK is initially membrane bound, whereas in case 5, the inactive FAK first needs to bind to the membrane, get activated, and then, in turn, activate RhoA, resulting in a higher sensitivity to the RhoA (un)binding rates.

**DISCUSSION**

Several nonintegral membrane proteins need to bind to the membrane to perform their biological function (17), and it has been shown theoretically (1,20,22,23) and experimentally (21–23) that cell signaling can be enhanced when these proteins interact with the plasma membrane. Particularly, the
formation of membrane receptor clusters and rafts by membrane-bound molecules helps concentrate the signal to specific areas of the cell membrane and amplify signals from the membrane (19–24). In this in silico study, we have shown that similar mechanisms are at play for the YAP/TAZ signaling pathway. More specifically, our results indicated that YAP/TAZ only translocated to the nucleus when the input signal FAK is membrane bound (cases 3–5). When FAK is activated in the cytoplasm, independent of whether RhoA is membrane bound or cytoplasmic, there was no YAP/TAZ nuclear translocation. Using a rigorous sensitivity analysis, we found that the membrane diffusion of the active forms of FAK and RhoA and their membrane binding dynamics were important regulators of YAP/TAZ nuclear translocation. Moreover, building on the work of others (27,28), we have confirmed that similar to 2D (3,9,27,84), YAP/TAZ nuclear translocation increases with cell spreading in three dimensions under particular conditions (28,76,85,86).

To study the role of membrane signal amplification in YAP/TAZ signaling, we developed a computational model for five types of FAK activation by distinguishing membrane-unbound (cases 1 and 2) and membrane-bound (cases 3–5) FAK. More specifically, our results indicated that YAP/TAZ only translocated to the nucleus when the input signal FAK is membrane bound (cases 3–5). When FAK is activated in the cytoplasm, independent of whether membrane bound or cytoplasmic, there was no YAP/TAZ nuclear translocation. Using a rigorous sensitivity analysis, we found that the membrane diffusion of the active forms of FAK and RhoA and their membrane binding dynamics were important regulators of YAP/TAZ nuclear translocation. Moreover, building on the work of others (27,28), we have confirmed that similar to 2D (3,9,27,84), YAP/TAZ nuclear translocation increases with cell spreading in three dimensions under particular conditions (28,76,85,86).
3–5) FAK activation. We observed that membrane localization of FAK contributes to a robust YAP/TAZ signal downstream. These results indicate that the membrane recruitment of FAK and anchoring to the membrane is important for YAP/TAZ signal amplification, similar to other signaling pathways (19,21,23–25). To explain the signal amplification through membrane localization, one needs to compare the dimensionality of the membrane (2D) with the one of the cytoplasm (3D). The reduced dimensionality of the membrane implies that the same number of active FAK molecules at the membrane (cases 3–5; Eq. 5) would translate into a higher initial active FAK concentration at the membrane compared to the cytosolic cases (cases 1 and 2; Eq. 5). Similarly, Schmick et al. (87) demonstrated that by considering the cytosol as a sphere and the plasma membrane as a shell around the cytosol, the concentration of a signaling effector initially diffusing in the cytosol would increase by \( \sim 1000 \)-fold if recruited and confined to diffusion on the membrane. Moreover, this type of increase in signaling molecule concentration at the membrane trumps the effects of reduced diffusion at the membrane (87). Interestingly, membrane localization of only RhoA (case 2) does not lead to a YAP/TAZ response. Indeed, for our cell size and initial number of FAK molecules, when RhoA and FAK do not colocalize at the membrane through (un)binding, it is impossible to trigger strong enough signals downstream to exceed the ROCK and mDia thresholds. Furthermore, we see an early attainment of peak YAP/TAZ concentration in case 3 compared to cases 4 and 5, which is consistent with the notion that cell signaling efficiency is dependent on the spatiotemporal organization of the signaling components (25,75,88). Therefore, not only membrane recruitment but also the particular signaling entity (in the specific pathway) being recruited are important.

Our in silico results underscore the need for taking into consideration membrane binding and unbinding dynamics of FAK and RhoA for YAP/TAZ signaling. It has been pointed out that the biological activity of several nonintegral membrane proteins is dependent on their membrane...
anchoring and thus their membrane binding and unbinding dynamics (17,18). However, despite the pivotal role played by membrane interactions in the activity of these proteins, it has been very difficult to quantify their membrane binding and unbinding rates experimentally (17). We have shown theoretically that YAP/TAZ nuclear translocation can be modulated by the membrane binding kinetics of RhoA and FAK. We observed an increase in the YTNF for higher inactive cytosolic RhoA binding rates. Indeed, the higher the binding rate, the more transfer of RhoA molecules to the membrane and the higher the chances of interaction with active FAK within the same membrane compartment. To investigate the effect of FAK binding and unbinding dynamics, we introduced a FAK activation rate instead of a fixed amount of initial active FAK. Interestingly, a high inactive cytosolic FAK binding rate increased the amount of inactive membrane-bound FAK available for activation at the membrane, thus leading to increased downstream activation and resulting in higher YTNF.

Our results agree with observations reported in literature (28,76,85,86) that the YTNF increases with cell spreading when RhoA is membrane bound. For example, in their study on the control focal adhesion by YAP signaling, Nardone et al. (89) showed that the YAP/TAZ nuclear signal increased with cell spreading, consistent with our results for cases 4 and 5. In their modeling work on the role of substrate stiffness, substrate dimensionality, and cell shape on YAP/TAZ signaling, Scott et al. (28) were able to predict that for a constant cell volume, YAP/TAZ would increase with substrate activation area in three dimensions (referred to as 2.XD) at medium (5.7 kPa) and high (7 GPa) stiffnesses. Their 2.XD corresponds to a 3D cell in which activation of FAK (and RhoA) is restricted to the base, similar to our simulation setup. Interestingly, although their approach is congruent with the need for RhoA to be membrane bound to obtain an increase in YAP/TAZ output with cell spreading (cases 4 and 5), their spatial model results do predict YAP/TAZ nuclear translocation when FAK is cytosolic and activated in the cytosol.
Oku et al. (55) have shown that 300 nM of dasatinib reduced the percentage of cells expressing nuclear YAP/TAZ from (402 m
model for 0–50 kPa. For similar FAK activation areas for case 5 on a standard cell of radius 16 m. The results show that stiffness sensing occurs in our model for 0–50 kPa. For similar FAK activation areas (402 m² vs. 415 m² in (28)), our stiffness sensing range (0–50 kPa) is lower than the one of Scott et al. (28) (0–100 kPa) and higher than in the 1D model of Sun et al. (27) (0–20 kPa). The differences in stiffness sensing ranges may arise from the fact that Scott et al. (28) also define a cytosolic stiffness, which is linked to the F-actin concentration, and relate it to the nuclear mechanics (lamin A activation).

Importantly, similar to our computational predictions, experimental literature as shown that the membrane localization of FAK or RhoA influences downstream YAP/TAZ signaling. For example, small molecules such as dasatinib, pazopanib, and lovastatin, which inhibit Rho GTPase prenylation and thereby prevent membrane localization, were found to reduce the nuclear localization of YAP (54,55,90). Oktay et al. (55) have shown that 300 nM of dasatinib reduced the percentage of cells expressing nuclear YAP/TAZ from ~80 to 5%. This corresponds to our predictions for case 1, in which there is no YAP/TAZ nuclear translocation. Interestingly, for case 3, in which RhoA is also cytoplasmic but FAK is membrane bound, the computational model does predict YAP/TAZ nuclear translocation. This difference may indicate that in the in vitro experiments mechanisms other than spatial location play a role in inhibiting YAP/TAZ translocation. In particular, by preventing membrane localization, the small molecules also reduce Rho GTPase activation, an effect we did not include in the model because we focused on the influence of localization only. Alternative splicing of FAK was shown to result in reduced focal adhesion formation, increased cytoplasmic localization of FAK, and reduced nuclear YAP (56,57), corresponding to the computational predictions in which the YAP/TAZ nuclear translocation is absent for cases 1 and 2 for the standard FAK activation scheme.

The results of this study, which summarize the role of membrane localization and binding and unbinding dynamics, diffusion, and cell spreading on YAP/TAZ nuclear localization, should be interpreted in the light of the following assumptions and limitations. Firstly, we do not account for the discrete nature of focal adhesions at the membrane, but rather assume that focal adhesion molecule activation and exchange happens in a continuous region in contact with the substrate. Furthermore, we assume that the signaling cascade is solely dependent on initial FAK activation, ignoring any signaling cross talk. Secondly, considering the short timescales that we model, we assume...
a constant amount of protein, thus ignoring potential production and degradation processes. Thirdly, we model a fixed nuclear volume and shape and a fixed distance of the nucleus from the center of the base of the cell, all of which are able to undergo dynamic changes with cell spreading. Finally, the current values of membrane exchange rates, especially for FAK, are estimated within ranges of scarcely available data. Experimental work needs to be done to obtain more accurate rate values within various cell spreading and environmental stiffness contexts, by using approaches such a fluorescence recovery after photobleaching. In summary, in this study we investigated the effect of FAK and RhoA membrane binding on YAP/TAZ signaling. We showed that FAK membrane binding can modulate and amplify the YAP/TAZ nuclear response. Moreover, we predicted an increase of YTNF with increased cell spreading, but only when FAK and RhoA are membrane bound. Future work should focus on the experimental verification of our predictions, namely on monitoring membrane interaction of FAK and RhoA and their effect on YAP/TAZ nuclear signal enhancement in relation to cell shape and dimensionality. By investigating the influence of membrane activation on downstream signaling, a motif common to many signaling pathways, this study contributed to an improved understanding of the design principles of signaling networks.

SUPPORTING MATERIAL
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AUTHOR CONTRIBUTIONS
K.S.E. designed and performed research, analyzed data, and wrote manuscript. R.C. designed and supervised research and gave feedback on manuscript. K.S. designed and supervised research and gave feedback on manuscript. J.d.B. supervised research and gave feedback on manuscript. A.C. designed and supervised research, gave feedback on manuscript, edited manuscript, and acquired financial support.

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