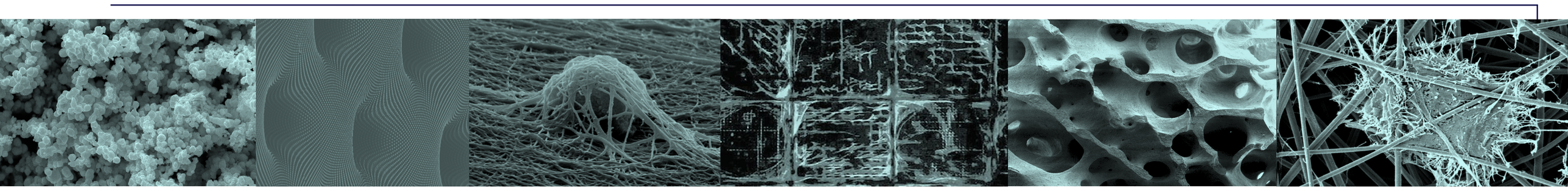

In silico modelling of force-dependent focal adhesion (dis)assembly

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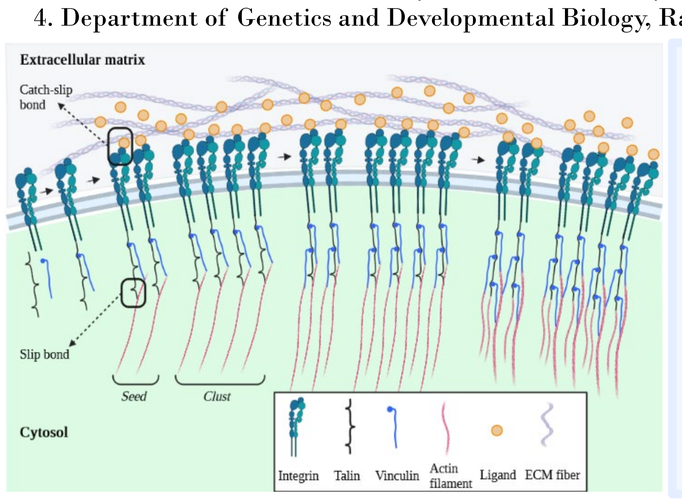

Background

- Mechanical properties of the extracellular matrix (ECM) influence important cellular processes such as morphogenesis, epithelial to mesenchymal transition (EMT), tumorigenesis, and migration. This occurs through mechanotransduction pathways facilitated by transmembrane proteins called integrins.
- Integrins bind to extracellular ligands and intracellular 'adaptor' proteins like talin and vinculin which bind to the actin cytoskeleton, forming a molecular clutch[1] forming a mechanical link between the cell and the ECM.
- Initially, integrin-talin precomplexes cluster at the cell membrane forming nascent adhesions (NAs). Hidden vinculin binding sites (VBS) on talin are uncovered when it is stretched, leading to additional vinculin recruitment[2]. Through this reinforcement, NAs undergo force-dependent maturation to focal adhesions (FAs) which are larger in size and can transmit larger forces[2].
- Here, **we present an ordinary differential equation based mechano-chemical computational model to investigate the force-dependent changes in the biochemical composition of adhesions.**

Methods

- The model has three main components: Integrins, talin, and vinculin (fig 1A).
- The substrate, and adaptor proteins talin and vinculin considered as Hookean springs (fig 1B), and actin binding is modelled implicitly.
- Two sizes of integrin clusters modelled – seeds (small clusters, S1a-S3a in fig 1B) and clusts (large clusters, C1a-C3a in fig 1B).
- Two reinforcement events – vinculin recruitment increases the force-carrying capacity of clutches.
- A hypothetical signal molecule accounts for the chemical events leading to NA disassembly – signal-dependent rate modification (SDRM).
  - $[signal]$ decreases monotonically, NA assembly and FA disassembly rates are reduced proportionally
- To account for spontaneous bond-rupture events, the actin-unbinding rate increases with the time a clutch remains actin-bound (t_{clutch}) – time-dependent rate modification (TDRM):
- Talin-actin slip bond off rate:

$$(1 + k_{sens} \cdot t_{clutch}) \cdot k_{slip_{UL}} \cdot e^{\frac{F_{clutch}}{F_{threshold}}}$$

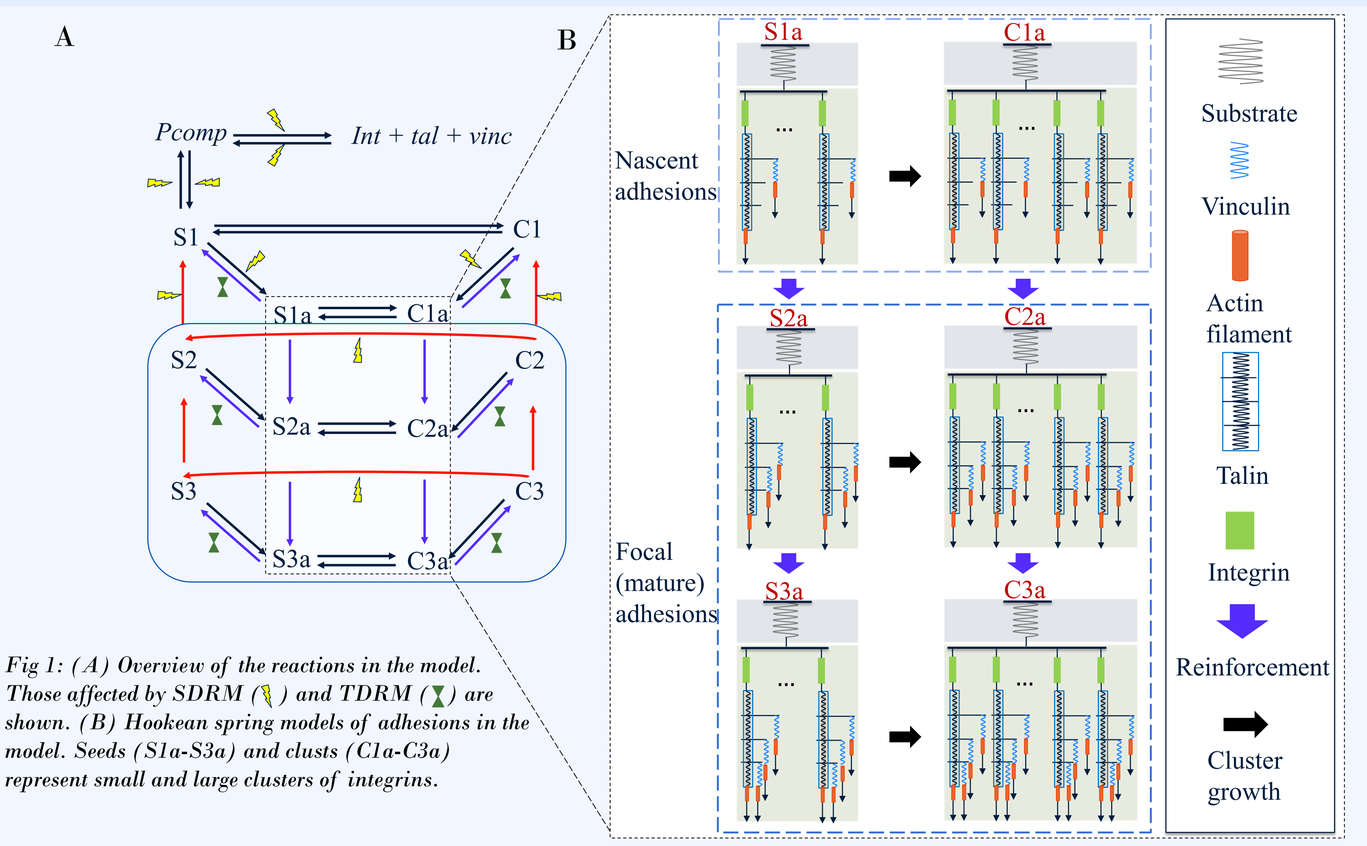

Fig 1: (A) Overview of the reactions in the model. Those affected by SDRM ( ) and TDRM ( ) are shown. (B) Hookean spring models of adhesions in the model. Seeds (S1a-S3a) and clusts (C1a-C3a) represent small and large clusters of integrins.

Results

- Moderate stiffnesses ($k_{sub} = 1\,pN/nm$, fig 2) result in highest adhesion maturation fraction (MF) and lowest actin retrograde velocity (highest traction force) (fig 3), in line with experimental studies[1] – said to be 'optimal' stiffness (OS).
- Oscillatory concentrations (fig 2) arise from bond-rupture events. For the baseline parameter values, the periods of oscillations for different substrate stiffnesses agree with previous computational and experimental studies[1,3].
- TDRM of actin-unbinding rates, SDRM of NA formation rates are essential for establishing an 'optimal' stiffness through mechanosensing.
- 20% decrease in vinculin availability results in ~9% increase in the actin retrograde velocity (or decrease in traction force) (fig 4)
- Increases in actin retrograde velocity, stiffness of talin reduce the OS and MF whereas increases in vinculin availability and actin-binding rates increase both OS and MF (fig 4)

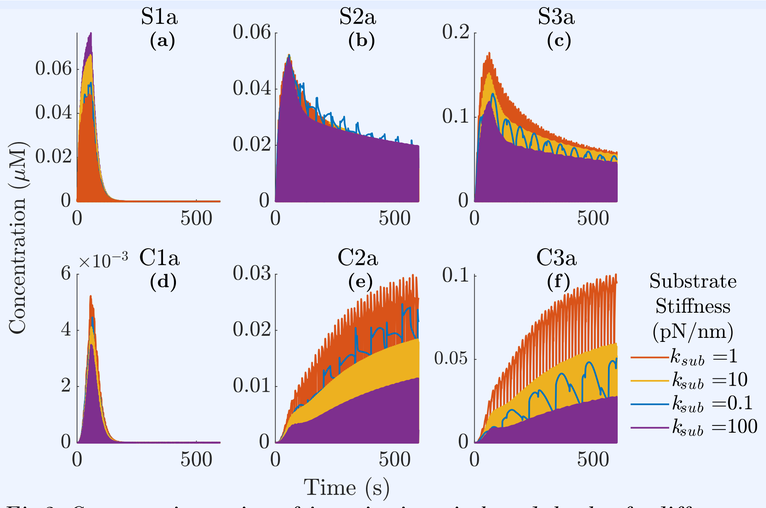

Fig 2: Concentration vs time of integrins in actin-bound clutches for different substrate stiffnesses

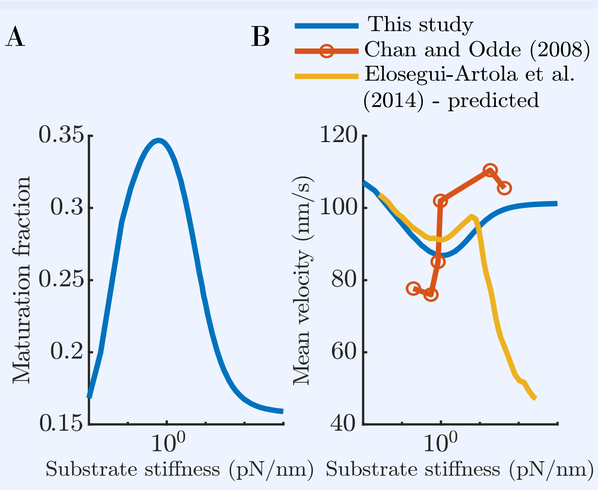

Fig 3: Maturation fraction (A) and mean actin retrograde velocity (B) vs substrate stiffness.

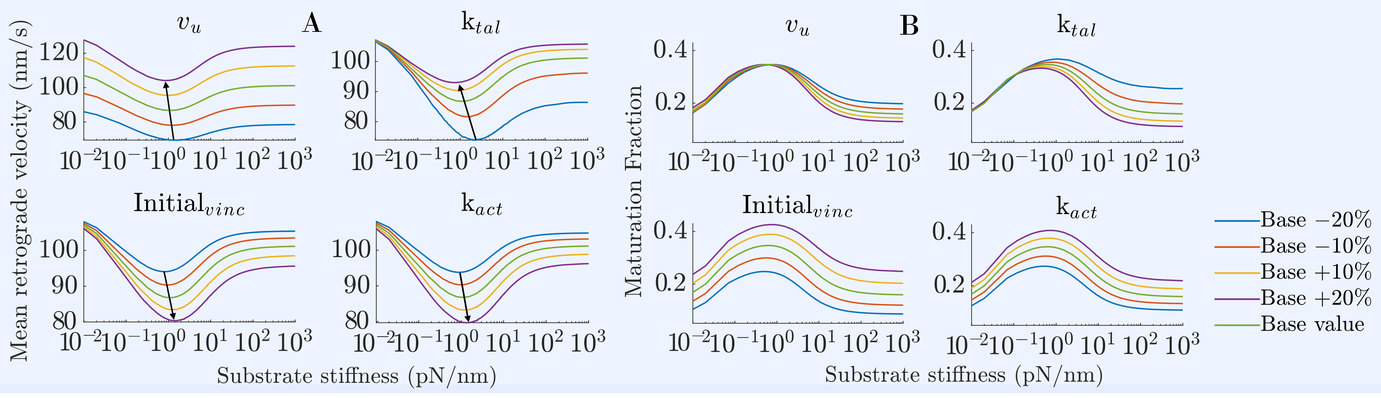

Fig 4: Mean actin retrograde velocity (A) and maturation fraction (B) curves vs substrate stiffness for a ±20% variation in parameter values.

Conclusions

- The model captures key trends in the maturation fraction of NAs, total force exerted by adhesions, actin retrograde velocity, periods of bond formation-rupture cycles, and stiffness sensitivity range, in excellent agreement with experimental studies. It bridges continuous and discrete aspects, namely concentrations and mechanical properties such as stiffness.
- This model framework can be used to predict that adhesion maturation fraction for a range of substrate stiffnesses for a variety of conditions by varying key parameters such as stiffness or availability of adaptor proteins, integrin clustering rates for instance.
- Kinetics of the generic signal molecule can be tuned to represent known signaling pathways that influence NA disassembly such as FAK phosphorylation[4], KANK-mediated talin-actin binding inhibition and microtubule-directed adhesion disassembly[5].

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