

FORCE-INDUCED UNFOLDING OF FIBRONECTIN IN THE EXTRACELLULAR MATRIX OF LIVING CELLS¹

[Michael L. Smith](#), [Delphine Gourdon](#), [William C. Little](#), [Kristopher E. Kubow](#), [Viola Vogel](#)

Department of Materials, ETH, Zurich, Switzerland.

INTRODUCTION: Whether mechanically unfolded fibronectin (Fn) is present within native extracellular matrix (ECM) fibrils is controversial. Fibronectin extensibility under the influence of cell traction forces has been proposed to originate from either the force-induced lengthening of an initially compact, folded quaternary structure as is found in solution (quaternary structure model where the dimeric arms of Fn cross each other), or from the force-induced unfolding of type III modules (unfolding model). Clarification of this issue is central to our understanding of the structural arrangement of Fn within fibrils, the mechanism of fibrillogenesis, and whether cryptic sites, which are exposed by partial protein unfolding, can be exposed by cell-derived force.

METHODS: In order to differentiate between these two models, two fluorescence resonance energy transfer (FRET) schemes to label plasma Fn were applied, with sensitivity to either compact-to-extended (arm separation) without loss of secondary structure or compact-to-unfolded conformations.

RESULTS: FRET studies revealed that a significant fraction of fibrillar Fn within a three-dimensional human fibroblast matrix is partially unfolded. Complete relaxation of Fn fibrils led to a refolding of Fn. The compactly folded quaternary structure with crossed Fn arms, however, was never detected within ECM fibrils.

DISCUSSION & CONCLUSIONS: We conclude that the resting state of Fn fibrils does not contain Fn molecules with crossed-over arms, and that the several-fold extensibility of Fn fibrils involves the unfolding of type III modules. This could imply that Fn might play a significant role in mechanotransduction processes.

REFERENCES: ¹M. Smith, D. Gourdon, W. Little, K. Kubow, R. Andresen Eguiluz, S. Luna-Morris, V. Vogel (2007) *PLoS Biol*, In Press.

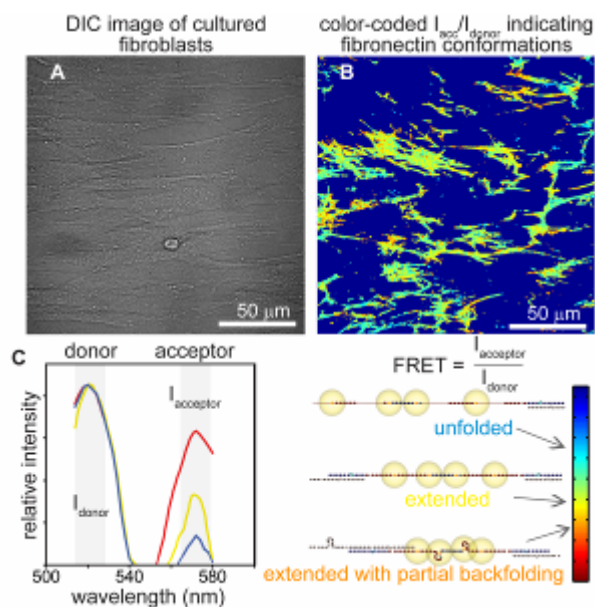


Fig. 1: Fibroblasts in cell culture (A) apply traction forces which lead to dynamic levels of strain within the ECM. Some regions (blue-green fibrils) are significantly unfolded, while other regions contain Fn with intact secondary/tertiary structure (yellow fibrils) and even quaternary structure (orange fibrils; B). Fluorescence Resonance Energy Transfer (FRET) is employed based on the increase in distance of covalently attached donor and acceptor pairs during the stretching of Fn in culture (B) or unfolding in denaturant in solution (C). Increased strain leads to decreased FRET. The proposed conformations of Fn corresponding to different FRET signals are shown (C).

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