The Molecular Dance of Fibronectin: Conformational Flexibility Leads to Functional Versatility

Review Article**Author(s):**

Mezzenga, Raffaele; Mitsi, Maria

Publication date:

2019-01-14

Permanent link:

<https://doi.org/10.3929/ethz-b-000317612>

Rights / license:

[In Copyright - Non-Commercial Use Permitted](#)

Originally published in:

Biomacromolecules 20(1), <https://doi.org/10.1021/acs.biomac.8b01258>

The molecular dance of fibronectin: conformational flexibility leads to functional versatility

Raffaele Mezzenga, Maria Mitsi**

Laboratory of Food and Soft Materials, ETH Zurich, Switzerland

ABSTRACT

Fibronectin, a large multimodular protein and one of the major fibrillar components of the extracellular matrix, has been the subject of study for many decades and plays critical roles in embryonic development and tissue homeostasis. Moreover, fibronectin has been implicated in the pathology of many diseases, including cancer, and abnormal depositions of fibronectin have been identified in a number of amyloid and non-amyloid lesions. The ability of fibronectin to carry all these diverse functionalities is through interactions with a large number of molecules, including adhesive and signaling cell surface receptors, other components of the extracellular matrix, and growth factors and cytokines. The regulation and integration of such large number of interactions depends on the modular architecture of fibronectin, which allows a large number of conformations, exposing or destroying different binding sites. In this review, we summarize the current knowledge regarding the conformational flexibility of fibronectin, with an emphasis on how it regulates the ability of fibronectin to interact with various signaling molecules and cell-surface receptors and to form supramolecular assemblies and fibrillar structures.

Introduction

1 Fibronectin is a secreted glycoprotein found in a soluble form circulating in plasma and in an insoluble
2 fibrillar form incorporated in the extracellular matrix of almost every cell type, where it functions as a major
3 substrate for cell adhesion and migration¹. Plasma fibronectin is produced by hepatocytes and it can become
4 incorporated into extracellular matrices, both *in vitro* and *in vivo*². Fibronectin has been implicated in a
5 large number of physiological processes³, including blood coagulation⁴, the uptake and consequent
6 clearance of blood-borne particles and bacteria by phagocytic cells (opsonic activity)⁵, wound healing and
7 tissue regeneration⁶, neovascularization⁷, and the orchestration of the complex morphogenetic movements
8 during embryonic development⁸. Its importance for organogenesis and tissue homeostasis can be
9 demonstrated by the fact that fibronectin null-mice die early during embryonic development due to severe
10 cardiovascular defects^{9,10}. On the other hand, abnormal depositions of fibronectin have been observed in a
11 number of fibrotic diseases^{11,12} and amyloidoses¹³. Moreover, dysregulation of cell adhesion and migration
12 on fibronectin has been implicated in tumor development and metastasis, and there is a large number of
13 studies focusing on fibronectin as a cancer biomarker, as well as a therapeutic target¹⁴.

14 The history of fibronectin is of interest, as it was discovered multiple times through different routes,
15 highlighting once again its wide range of functions. The first report of fibronectin (at the time called cold-
16 insoluble globulin) dates 1948, as a part of the first systematic study to fractionate the proteins of the
17 plasma¹⁵. However, it did not attract much attention, and it was not until the 1970s that studies for its
18 purification and characterization were performed¹⁶, in parallel with independent studies of plasma proteins
19 with opsonic activity (α 2-macroglobulin)¹⁷. At about the same time, research from a different direction
20 identified insoluble proteins on the surface of cultured cells (named fibroblast surface antigen and large
21 external transformation-sensitive protein), which were lost during oncogenic transformation and exhibited
22 cell adhesive properties^{18,19}. Gradually, it became evident that all of these diverse functions were performed
23 by the same protein, sparking renewed interest in the research community. The protein was named
24 fibronectin²⁰, and, as has been noted by Jan McDonagh, it was ‘transformed from a protein in search of a
25 function to a protein with possibly too many functions’²¹.

26 Intensive sequencing, biochemical and biophysical studies performed in the 1980s and 1990s to
27 characterize fibronectin, yielded a wealth of information regarding its molecular architecture and possible

1 modes of function. A picture of fibronectin as a multimodular protein, possessing many different binding
2 sites, and a significant degree of conformational flexibility emerged. Research during the last two decades,
3 taking advantage of the technological advances in molecular biology, imaging and structural techniques,
4 has provided further insights in the complexity of the structure and function of fibronectin, while new
5 functions continue to be revealed. Since its discovery more than 50 years ago, our ideas about fibronectin
6 have evolved: from a structural component keeping tissues together, it is now considered as a dynamic
7 molecule orchestrating the way biochemical and mechanical stimuli are presented to and interpreted by the
8 cells. However, despite decades of research, our understanding of the underlying molecular mechanisms
9 remains incomplete. This review aims at summarizing our current knowledge on the conformational
10 flexibility of fibronectin, with an emphasis on how it regulates the ability of fibronectin to interact with
11 various signaling molecules and cell-surface receptors and to form supramolecular assemblies and fibrillar
12 structures, and to formulate open questions with the potential to drive the field forward.

14 Modular architecture and conformational flexibility

15 Fibronectin, isolated from both plasma and the extracellular matrix, is a large dimeric protein encoded
16 by one gene, and it is composed of two very similar but not identical polypeptide chains, each with a
17 molecular weight of 250-270 kDa, linked by two disulfide bridges near their C-termini in an antiparallel
18 fashion²². One of the prominent features of fibronectin is its ability to bind to cells through specific cell-
19 surface receptors (integrins)²³, as well as to a large number of other biomacromolecules, including
20 collagen²⁴, heparin²⁵, fibrin²⁶, and even DNA²⁷. These binding capacities can be localized in distinct
21 functional domains that can be released as stable fragments by proteolysis, suggesting a modular
22 architecture²⁸. Indeed, spectroscopic, calorimetric and denaturation studies²⁹⁻³² of both intact fibronectin
23 and its fragments showed that it is composed of a large number of independent structural modules, classified
24 into three different categories based on sequence homology: type I, type II, and type III. Later structural
25 studies have shown that each module has a consensus fold, with antiparallel β -strands of a well-defined
26 topology³³. Each fibronectin chain is composed of 12 type I, 2 type II and at least 15 type III modules,
27 connected via loops of various sizes, flexibilities and orientations. Alternative splicing can result in the

inclusion of one or two additional type III modules (termed EDA and EDB), or of a variable sequence (known as V or IIICS), accounting for the variability observed between the two fibronectin chains, as well as between plasma and cellular fibronectin³⁴. The independent functional units along the fibronectin chain, which exhibit distinct binding capacities and the ability to be released by proteolytic digestion without structure or function loss, are formed by groups of different modules³³. The major fibronectin functional domains are summarized in Figure 1, although additional subdivisions are possible. Starting from the N-terminus, I₁-I₅ binds to heparin and fibrin; it also interacts with bacteria, contains a major transglutaminase cross-linking site and is critical for the incorporation of fibronectin into the fibrillar extracellular matrix. I₆-I₉ forms the collagen/gelatin binding domain. Modules III₂-III₁₁ are traditionally considered as the cell-binding domain, although the major integrin binding sites have been localized in III₉-III₁₀ and the remaining modules are involved in fibrillogenesis. III₁₂-III₁₅, also known as Hep2, is the major heparin-binding site of fibronectin, contains additional integrin binding sites, and is a promiscuous binding site for a multitude of growth factors. I₁₀-I₁₂ is the second fibrin-binding site of fibronectin.

Figure 1. The multimodular architecture of fibronectin. Schematic representation of modules type I (purple), type II (green) and type III (red) constituting one chain of fibronectin. The alternatively spliced

1 type III modules EDA and EDB, as well as the alternatively spliced region V are shown in a lighter color.
2 The disulfide bridges linking the two chains at their C-termini in an antiparallel fashion are also shown.
3 Several modules are forming functional domains displaying distinct binding affinities, and they can be
4 released as stable fragments by proteolysis. The major proteolytic sites and the respective proteases are
5 shown, together with the molecular weights of the resulting fragments (bottom), and some of their binding
6 affinities (top).

7
8 Although in the schematic representation of fibronectin the modules are shown in a linear, end-to-end
9 arrangement, intermodular interactions allow for a high degree of conformational flexibility. Using
10 overlapping and progressively shorter fragments spanning the major fibronectin functional domains,
11 differential scanning calorimetry (DSC) studies^{35–37} identified strong interactions between several adjacent
12 modules: I₂-I₃, I₄-I₅, II₁-II₂, III₄-III₅, III₈-III₉, III₁₃-III₁₄, as well as between non-adjacent modules: I₆-I₇, III₃-
13 III₆ and III₇-III₉. Such interactions can be stabilizing or destabilizing for the individual modules. For
14 example, III₈ is stabilized by its interactions with III₉ (increased melting temperature), whereas III₇ is
15 destabilized by III₉ (decreased melting temperature)³⁶. The molecular components of such underlying
16 interactions are not known, as is their functional significance. It is interesting to note however, that these
17 arrangements result in an alternating pattern of thermostable and thermolabile structural units along the
18 fibronectin chain, at least for the regions I₆-I₉ and III₂-III₁₁. Solution nuclear magnetic resonance (NMR)
19 structures of module pairs have confirmed some of these interactions^{38–40}, and provided evidence for
20 additional interactions, such as the one between I₆ and II₁^{41,42}.

21 Sedimentation equilibrium studies and solid-phase binding assays have identified further interactions
22 between distal parts of fibronectin⁴³. Some of these self-association sites can operate within one chain or
23 between chains, folding the fibronectin molecule upon itself and maintaining a compact configuration. It
24 has been postulated⁴³ that interactions between modules III₂-III₃ from one chain with modules III₁₄-III₁₅
25 from the other chain keep fibronectin in solution in a compact configuration. These interactions are
26 primarily electrostatic in nature, with III₂-III₃ having an excess negative charge and III₁₂-III₁₄ an excess

1 positive charge, and they are significantly enhanced at low ionic strength⁴³. Indeed, fibronectin adopts a
2 more compact configuration in solutions of low ionic strength, a phenomenon that critically depends on the
3 presence of modules III₁₂-III₁₄. When these modules were removed or replaced with type III modules from
4 tenascin, fibronectin adopted a much less compact conformation, which was no longer sensitive to the ionic
5 strength⁴³.

6 When considering self-association sites within the fibronectin molecule, it is very important to keep in
7 mind that the presence of neighboring modules can stabilize or destabilize certain interactions or impose
8 additional constraints through distal interactions^{35,36}. This can best be exemplified by the efforts to elucidate
9 the structure of the collagen-binding domain of fibronectin (I₆-II₁-II₂-I₇-I₈-I₉). Two independent studies of
10 the I₆-II₁ module pair by solution NMR^{41,42} reported a weak interaction via a small intermodular interface,
11 a flexible linker that does not appear to be involved in stabilizing or orienting the modules, and a significant
12 degree of freedom, although a preferred orientation between the two modules was observed. Similarly, the
13 pair II₁-II₂ showed no well-defined interface and a high degree of flexibility⁴⁰. However, a completely
14 different picture emerged from the structure of the slightly larger fragment I₆-II₁-II₂ by solution NMR⁴⁴. The
15 limited interface between I₆ and II₂ was disrupted in the presence of the additional module. More strikingly,
16 an extensive hydrophobic interface (868 Å²) was observed between I₆ and II₂, giving a hairpin topology to
17 the entire domain, which may increase accessibility to the domain I₁-I₅, facilitating fibrillogenesis. A very
18 similar arrangement was observed by X-ray crystallography of the I₆-II₁-II₂-I₇ domain⁴⁵, revealing a very
19 compact pyramidal shape with I₆-II₁-II₂ forming the base triangle and I₇ the projecting tip, making contacts
20 with both I₆ and II₂. The I₆-II₁-II₂ modules in the crystal structure adopted a very similar structure as in
21 solution but with a better-defined interface between II₁ and II₂. Yet, the crystal structure of the entire
22 collagen-binding fragment (I₆-II₁-II₂-I₇-I₈-I₉) showed a somewhat different picture⁴⁶. A curved, elongated
23 shape (40 x 40 x 82 Å) with the well-defined I₆-II₂ interface forming its N-terminal lobe, module II₁ at the
24 center and modules I₇-I₈-I₉ adopting a super-domain 8-stranded antiparallel β-sheet configuration, forming
25 the C-terminal lobe of the domain. In this structure, I₈ exhibits significant deviations from the consensus
26 type I fold. However, the high concentration of Zn²⁺ that was used as the crystallization agent may be
27 responsible for the unusual arrangement of modules I₇-I₈-I₉, as 4 Zn atoms were observed interacting with

1 I₈ in the crystal structure. Indeed, such rearrangements were not observed in the crystal structure of a
2 complex between isolated I₈-I₉ and collagen⁴⁷. The physiological relevance of Zn²⁺ in regulating fibronectin
3 conformation and collagen binding is uncertain, although it demonstrates the capacity of I₈ to undergo
4 drastic conformational changes.

5 The nature of the intermodular interactions regulating the structure of fibronectin in solution is complex
6 and highly dynamic. Factors such as pH, ionic strength, glycerol, heparin, mechanical stretching and
7 adsorption at surfaces of different chemistries, can affect fibronectin conformation^{43,48–51}. This suggests the
8 possibility that fibronectin has the ability to adopt many different configurations between a compact and a
9 fully extended structure, supported by intermodular interfaces of different flexibilities and distal
10 interactions that introduce deviations from a strictly linear end-to-end modular arrangement^{52,53}. Thus,
11 different modules can be brought together through conformational changes to regulate not only the global
12 shape of the molecule but also its functionality: cryptic binding sites can be exposed, existing binding sites
13 destroyed, and new binding sites formed.

14

15 Interactions between fibronectin and heparin

16 As we will discuss in detail in this review, the linear polysaccharide chains of heparin and heparan
17 sulfate are components of the extracellular microenvironment with the ability to influence
18 fibronectin conformation and function. Heparan sulfate chains are covalently attached to proteoglycans,
19 a large family of proteins found either cell-surface bound or secreted in the extracellular matrix⁵⁴. Heparin
20 on the other hand, is not attached to any proteoglycans but is secreted exclusively by mast cells,
21 specialized cells of the immune system, which often accumulate in areas of tissue injury^{55,56}. Heparin
22 shares the same backbone structure with heparan sulfate but exhibits a higher degree of sulfation, and is
23 often used as a model of heparan sulfate to identify heparan sulfate binding sites on proteins⁵⁷. Prior to
24 discussing the effect of heparin/heparan sulfate on fibronectin conformation, this chapter will focus on the
25 complex interactions between heparin and fibronectin, involving multiple binding sites, which are
26 themselves subject to conformational regulation, yielding results that are sometimes conflicting and
27 difficult to interpret, as we discuss below.

1 There are at least three heparin-binding sites on fibronectin: Hep1 at the N-terminal part of the
2 molecule (I₁-I₅), Hep2 at the C-terminal (III₁₂-III₁₄), and Hep3 at the central part of the molecule (III₄-
3 III₆). Solid-phase binding assays^{58,59}, as well as binding in solution⁶⁰, have shown that Hep2 exhibits
4 a very strong affinity for heparin, whereas Hep1 is only weakly bound. Moreover, heparin binding to
5 Hep1 is inhibited by Ca²⁺, whereas that on Hep2 is Ca²⁺-insensitive⁶¹. Hep3 also shows low affinity
6 for heparin⁶². Thus, Hep2 is considered the major heparin-binding site of fibronectin.

7 However, quantitative studies have shown that the actual affinity of Hep2 for heparin is rather low:
8 a dissociation constant K_d of ~5 μM was determined by plasmon surface resonance (SPR) on
9 immobilized heparin⁶³. A similar K_d value (~2 μM) was reported for the interaction of heparin with
10 full-length fibronectin in solution by fluorescence polarization measurements⁶². The stoichiometry
11 of the interaction identified in this study was 2 heparin binding sites per Hep2 domain (4 heparin
12 sites on a fibronectin dimer). However, mapping the heparin binding sites on fibronectin by single-
13 molecule AFM using colloidal gold conjugated to heparin⁶⁴ showed, somewhat unexpectedly, that the
14 majority of heparin binding events occurred close to the N-terminus, suggesting that under these
15 conditions, Hep1 bound heparin stronger than Hep2. Similarly, a study of the mechanical properties
16 of the heparin-fibronectin interactions by single-molecule dynamic spectroscopy⁶⁵ showed one
17 major type of fibronectin-heparin interactions (i.e., one binding site) but with several fibronectin-
18 heparin pairs forming simultaneously. The fact that the binding events were Ca²⁺ sensitive points to
19 the Hep1 being the binding partner.

20 These conflicting data concerning the affinity of the various heparin binding sites of fibronectin
21 could be reconciled if one considers the possibility that the heparin binding sites themselves are
22 subject to conformational regulation. Studies from one of the authors⁶⁶ have shown that binding
23 assays immobilizing heparin or fibronectin do not yield equivalent results. Whereas the entire
24 amount of soluble fibronectin can be precipitated by heparin-agarose, immobilized fibronectin
25 possesses very few heparin binding sites (~1 bound heparin molecule per 20 fibronectin molecules)
26 but of high affinity (K_d ~20 nM). The isolated Hep2 domain shows a similar behavior. The idea that

1 the Hep2 binding site is not readily available for heparin binding on surface-adsorbed fibronectin
2 can also explain the results of the aforementioned AFM and single-molecule dynamic spectroscopy
3 studies. In both these studies, fibronectin most likely adopts an extended configuration, through
4 adsorption on a hydrophilic surface⁶⁴ or the use of a spacer to immobilize it on the AFM tip⁶⁵, and is
5 able to bind heparin mostly via the Hep1 domain. On the other hand, when fibronectin adopts a more
6 compact configuration through adsorption on a hydrophobic surface, both Hep1 and Hep2 sites
7 appear to be blocked⁶⁶. Therefore, heparin binding to fibronectin is in general conformation
8 sensitive. Indeed, it was shown that in solid-phase binding assays, heparin binds more efficiently to
9 the isolated Hep2 domain than in a fusion construct between III₈-III₁₀ and Hep2, i.e., the neighboring
10 modules mask the heparin-binding site⁶⁷.

11 Heparin binding on Hep2 occurs primarily on module III₁₃⁶⁸, although some studies have shown
12 that III₁₄ also contributes to the interaction⁶⁹. Inclusion of the alternatively spliced EDA and V regions
13 to the N- and C-terminal of Hep2 respectively, does not interfere with heparin binding⁶⁹. However,
14 peptides isolated from III₁₃ containing the critical sequences with heparin-binding capacity often fail
15 to bind heparin^{70,71}. It has been proposed that the spatial arrangement of these residues is very
16 important for determining heparin binding and affinity^{70,72}. Indeed, the basic residues on III₁₃ are
17 clustered into a cationic 'cradle', whose spatial arrangement is the essential factor for heparin
18 binding⁷². Supporting this hypothesis is the observation that although III₁₄ is more basic than III₁₃,
19 its affinity to heparin is very low, correlating with a wider distribution of the basic residues over its
20 surface⁷². This further supports the hypothesis that heparin binding to Hep2 is conformation
21 sensitive.

22 With respect to the requirements of heparin for binding to fibronectin, it was shown that the chain
23 size and its degree of N-sulfation are critical determinants⁷³. The effect of size seems to be more
24 important for the binding of small heparin oligosaccharides (2-6 kDa) to fibronectin, whereas for
25 heparin populations of higher molecular weight (5-30 kDa) did not play a role⁶². The prominent role
26 of N-sulfation has also been observed with heparan sulfate chains: domains of the chain that exhibit

1 a high degree of sulfation (S-domains) bind strongly to fibronectin⁷⁴⁻⁷⁶. This last observation has
2 interesting implications concerning the physiological relevance of studies investigating heparin-
3 fibronectin interactions and their consequences for fibronectin structure and function. The results of
4 such studies may well reflect mechanisms operating in sites of mast cell accumulation, where heparin
5 secretion takes place, or alternatively, they may apply to local interactions between fibronectin and
6 the highly sulfated 'heparin-like' S-domains of heparan sulfate proteoglycans in the extracellular
7 matrix.

8

9 Heparin-mediated exposure of cryptic growth factor binding sites

10 AFM studies from one of the authors have shown that interactions between fibronectin and heparin
11 or heparan sulfate chains mediate a conformational change on fibronectin, from a compact to a more
12 extended configuration, which exposes cryptic Vascular Endothelial Growth Factor (VEGF) binding
13 sites on Hep2⁷⁷. For the sake of simplicity, we will use the term heparin instead of heparin/heparan
14 sulfate to describe this phenomenon. A similar heparin-mediated conformational change, which
15 exposed a specific antibody epitope on Hep2 while destroying another one, was observed by quartz
16 crystal microbalance with dissipation (QCMD) in a different study⁷⁸. Moreover, this conformational
17 change reduces the affinity for heparin, leading to its dissociation and allowing it to diffuse and
18 interact with neighboring fibronectin molecules, altering their conformation as well. In this way,
19 substoichiometric amounts of heparin are sufficient to alter the conformation of a large number of
20 fibronectin molecules within the matrix, acting in a catalytic way (Figure 2)⁶⁶. To our knowledge, this
21 is the only report of a macromolecule acting as a structural catalyst. According to this model, the open
22 conformation of Hep2 is thermodynamically more stable, but separated from the compact
23 configuration by a large kinetic energy barrier, associated with strong intermodular interactions.
24 Heparin binding interferes with these interactions, reducing the energy barrier, and facilitating the
25 transition to the open conformation⁶⁶. Indeed, earlier calorimetric studies have shown that heparin
26 stabilizes the isolated III₁₂-III₁₄ modules, through breakage of the interactions between III₁₂ and III₁₃-

1 III₁₄³⁷. In fact, calorimetric studies have shown that interactions with heparin increase the melting
 2 temperature of full-length fibronectin as well, exerting a stabilizing effect⁷⁹.

3
 4 **Figure 2. The catalytic mechanism of heparin altering fibronectin conformation.** Interactions between
 5 heparin (wavy line) and fibronectin induce a conformational change from a compact (gray) to an extended
 6 (red) configuration, which reduces the affinity for heparin, allowing for its dissociation, diffusion and
 7 binding to neighboring fibronectin molecules. In this way, substoichiometric amounts of heparin can alter
 8 the conformation of the entire fibronectin matrix, acting as a structural catalyst. Three catalytic cycles are
 9 shown. One of the functional consequences of the heparin-catalyzed conformational change on fibronectin
 10 is the exposure of cryptic growth factor binding sites on the Hep2 (III₁₂-III₁₄) domain of fibronectin⁶⁶.

11
 12 The binding site exposed on Hep2 by the effect of heparin can be recognized by several members and
 13 isoforms of the VEGF family, but not by Epidermal Growth Factor (EGF) or Fibroblast Growth Factor
 14 (FGF)-2⁷⁷. However, the open Hep2 binds stronger Platelet-Derived Growth Factor (PDGF)-AA, and
 15 enhances its potential to direct mesoderm migration during embryonic development⁸⁰. An SPR study⁸¹ using
 16 immobilized modules III₁₂-III₁₄ showed that this domain of fibronectin has a remarkable ability to bind to
 17 a large number of growth factors: several members of the VEGF, PDGF, FGF, Bone Morphogenetic Protein

1 (BMP), Nerve Growth Factor (NGF), and Insulin Growth Factor Binding Protein (IGFB) families,
2 Transforming Growth Factor (TGF)- β 1, Heparin-binding EGF (HB-EGF), Hepatocyte Growth Factor
3 (HGF), and Connective Tissue Growth Factor (CTGF). Not all of these growth factors share the same
4 binding site on III₁₂-III₁₄, as suggested by cross-competition experiments. Moreover, treatment of III₁₂-III₁₄
5 with heparin increases the binding of some but not all growth factors, in full agreement with the results of
6 the study demonstrating the catalytic action of heparin on fibronectin⁷⁷. Based on these observations, we
7 hypothesize that regulatory mechanisms must exist that control the presentation of specific growth factor
8 binding sites on the Hep2 domain of fibronectin, relying, at least partly, on the conformational flexibility
9 of fibronectin and the ability of heparin to catalyze local structural rearrangements on this domain.

10 Binding of growth factors on fibronectin may be an important factor regulating their signaling potential
11 and biological output. Indeed, binding of IGFBP-5⁸², IGFBP-3⁸³, HGF⁸⁴, CTGF⁸⁵ and VEGF⁸⁶ to
12 fibronectin, and particularly to the Hep2 domain, enhances or at least regulates their activity. This can be
13 partly explained by indirect effects, such as sequestration in the matrix that alters their bioavailability and
14 generates gradients that influence the cellular response^{87,88}, but alternative, not necessarily mutually
15 exclusive, mechanisms could also play a role. Matrix-bound growth factors could alter their cognate
16 receptor trafficking, recruit co-receptors that alter their signaling output, or induce a distinct receptor
17 conformation that activates different signaling pathways. For example, it has been shown that immobilized
18 VEGF at the same effective amounts as soluble VEGF and without the formation of gradients activates
19 distinct downstream signaling pathways^{89,90}. Deciphering the mechanisms via which fibronectin in the
20 extracellular matrix regulates the signaling potential of growth factors will have an impact not only for
21 basic science but also for therapeutic and tissue engineering applications.

22 Although Hep2 has been recognized as a promiscuous growth factor binding domain of fibronectin,
23 additional domains have been identified that interact with specific growth factors. HGF can also bind at the
24 N-terminal domain of fibronectin (I₁-I₉)⁸⁴ and PDGF-BB interacts with both the Hep2 and with module
25 III₁⁹¹. Notably, III₁ contains two binding sites for PDGF-BB in its C-terminal sequence⁹². Hep2 also contains
26 two PDGF-BB binding sites, which share sequence similarity with these on III₁⁹². These PDGF-BB binding

1 sites on fibronectin are critical for fibroblast survival^{91–93}. It will be interesting to investigate whether these
2 sites are able to bind other growth factors as well.

3 It should be noted that the extension of the Hep2 fibronectin domain by heparin and the ensuing exposure
4 of certain growth factor binding sites cannot be induced by mechanical forces, although the heparin-
5 mediated conformational change can be modulated by the extent of fibronectin stretching (Mitsi M.,
6 unpublished data). Similarly, mechanical forces could not expose a certain heparin-induced antibody
7 epitope on Hep2 but stretching reduced the heparin effect⁷⁸. This demonstrates the exquisite specificity and
8 intricacy of the mechanisms that regulate fibronectin conformation and offers an explanation of how a
9 single domain of fibronectin can sustain so many different binding capacities in a functional way.

10 Furthermore, different parts of fibronectin, and the binding sites therein, may be differentially susceptible
11 to biochemical or mechanical factors. Indeed, the N-terminal part of fibronectin (I₁-III₁) seems to be
12 particularly prone to stretch-induced conformational changes: the binding sites for bacterial adhesins⁹⁴ and
13 collagen⁹⁵, as well as a specific antibody epitope on I₉-III₁⁹⁶, are destroyed upon stretching, whereas the
14 self-association with the N-terminal fragment of fibronectin (I₁-I₉) is enhanced upon stretching⁹⁶. Moreover,
15 a recent study from one of the authors has identified a binding site for the cytokine Interleukin (IL)-7 on
16 the N-terminal domain of fibronectin, which interacts stronger with IL-7 upon fibronectin stretching⁹⁷.

17

18 Conformation-sensitive fibronectin-integrin interactions

19 Fibronectin exerts its cell adhesive properties by binding to integrins, a large family of
20 heterodimeric transmembrane cell-surface receptors, composed of two subunits, α and β ⁹⁸. There
21 are at least 20 different $\alpha\beta$ combinations, each with distinct binding affinities for proteins of the
22 extracellular matrix and signaling properties. Although fibronectin recognizes at least 10 different
23 integrin heterodimers, $\alpha_5\beta_1$ is considered its major cell-surface receptor, responsible for a large
24 number of its functions, including fibrillogenesis²³. As we will discuss in this section, the fibronectin-
25 integrin interactions are conformation sensitive: integrin binding requires a precise fibronectin
26 conformation, while it can itself cause conformational changes on fibronectin, altering the strength
27 of the interaction, as well as the intermolecular interface.

1 The major integrin binding site on fibronectin contains the RGD sequence in module III₁₀, which is
2 located in a loop protruding from the main body of the module, exhibiting significant conformational
3 flexibility^{99,100}. ¹⁵N relaxation measurements show that this loop can undergo extensive movements
4 at the ps-ns time scale, although it appears relatively immobile at the μ s-ms time scale¹⁰¹. Thus, it can
5 search and dock at the appropriate integrin interface. In a crystal structure of III₁₀¹⁰², the RGD loop
6 appears disordered, in agreement with the hypothesis that it exhibits significant flexibility.

7 Although the RGD sequence is the main determinant of fibronectin-integrin interactions, early
8 studies identified a sequence N-terminal to RGD, which acts synergistically with RGD for $\alpha_5\beta_1$
9 binding¹⁰³⁻¹⁰⁵. This synergy site has been localized on III₉ and its minimal sequence requirement is
10 the peptide PHSRN^{106,107}. Whereas the RGD loop is recognized by both α_5 and β_1 integrin subunits,
11 the synergy site binds mainly to the α_5 subunit¹⁰⁸⁻¹¹⁰. Thus, the two sequences act in a cooperative
12 manner to engage the integrin, and their positioning and relative orientation in fibronectin are
13 essential for determining the specificity and strength of the interaction. Solution NMR studies of III₉-
14 III₁₀ showed a lack of a well-defined interface, as well as of a preferred orientation between the
15 modules, while ¹⁵N relaxation time measurements showed significant flexibility of the linker between
16 them at the ps-ns time scale^{111,112}. Such interdomain flexibility would allow for movements that
17 would bring the RGD and synergy loops in the proper position for integrin binding. The crystal
18 structure of III₇-III₁₀, the first X-ray crystallographic study of linked type III modules¹¹³, revealed an
19 extended 140 Å long array, resembling a beads-on-a-string arrangement, with a pseudotwofold
20 relationship between adjacent domains. The RGD loop was resolved in this structure because it was
21 stabilized by crystal contacts. It protrudes from the structure and is free from contacts from the rest
22 of the molecule, and free to participate in integrin binding. The angles between modules vary; the
23 smallest is that between III₉ and III₁₀ (12°), which brings the RGD and synergy loops in high
24 proximity. The interface between III₉ and III₁₀ is one of the smallest observed in fibronectin so far,
25 supporting the idea that it is a point of high flexibility along the fibronectin chain.

1 Several observations suggest that both the flexibility of the RGD and synergy loops, as well as the
2 flexibility around III₉-III₁₀, are able to create multiple integrin-fibronectin interfaces, with distinct
3 functions. Firstly, not all integrins require the synergy site for binding to fibronectin^{114,115}. In fact,
4 differentially activated integrins will bind III₉-III₁₀ through distinct sites and with distinct
5 strengths^{114,116}. Indeed, mapping the synergy site by mutagenesis¹¹⁷ has shown that it includes a
6 much more extensive interface than the originally proposed PHSRN peptide, which is widely
7 distributed over the surface of III₉, and can accommodate different binding interfaces. Interestingly,
8 mutations in the synergy site that impair its functionality also impair its stability and folding¹¹⁸. This
9 was rather unexpected as PHSRN lies on a solvent exposed loop. As III₉ is chemically less stable than
10 III₁₀, it was suggested that an additional role of the synergy site is to stabilize III₉. Therefore, any
11 factor which could increase the global stability of III₉ would overcome the need for the synergy site
12 for integrin recognition by fibronectin.

13 Moreover, mechanical forces transmitted through the actin cytoskeleton may act as a mechanical
14 switch, transforming the $\alpha_5\beta_1$ -fibronectin complex from a relaxed into a tensioned state¹¹⁹. The two
15 states are characterized by distinct intermolecular contacts: the relaxed state depends primarily on
16 RGD binding, whereas the tensioned state involves both the RGD and synergy sites. Mechanical
17 unfolding may provide an additional level of conformational regulation of integrin-fibronectin
18 binding. Steered molecular dynamics simulations¹²⁰ support a hypothesis whereby mechanical
19 forces can unfold III₉-III₁₀ in three sequential steps that change the distance and relative orientation
20 between the two modules. The simulations predict that at low levels of stretch, a pre-stretched state
21 occurs, in which the distance between PHSRN and RGD increases but is still sufficient for $\alpha_5\beta_1$
22 binding. At intermediate stretch levels, a de-coupled state further increases the distance between the
23 two states and also changes their orientation, disrupting cooperative $\alpha_5\beta_1$ binding but still sustaining
24 binding of individual integrins on the two sites. Finally, high levels of stretch result in a completely
25 unfolded state, where both binding sites are destroyed. Thus, mechanically regulated fibronectin
26 conformation could affect integrin binding, as well as the requirements for the synergy site.

Fibronectin matrix assembly

Many cell types have the ability to assemble exogenously added or endogenously produced fibronectin into a fibrillar meshwork¹²¹, in a stepwise process that requires an intact actin cytoskeleton and the ability of the cell to contract and apply tension¹²². Thus, newly assembled fibronectin fibrils coalign with bundles of actin filaments in the cytoskeleton and with focal adhesions¹²³. Moreover, disruption of the actin cytoskeleton inhibits fibronectin matrix assembly^{124–126}. At the same time, fibronectin fibrillogenesis depends on the interactions between $\alpha_5\beta_1$ and fibronectin. Antibodies blocking either the RGD sequence on fibronectin¹²⁷ or the $\alpha_5\beta_1$ integrin^{128,129} inhibit fibronectin incorporation into the matrix, localization of α_5 into focal adhesions, and cell adhesion. The essential element in this process is the ability of the integrin to engage its ligand, as deletion of the cytoplasmic domain of α_5 ¹³⁰ or substitution of it with that of other integrins¹³¹ has no effect. During the early steps of fibronectin fibrillogenesis, $\alpha_5\beta_1$ bound to fibronectin translocates from the cell periphery towards the cell center, pulling on the fibronectin molecules and induces fibril formation¹³². Mapping the traction forces generated by the adherent cell showed a strong positive correlation between the direction of the pulling force exerted by the cell and the direction of fibronectin fibrillogenesis¹³³. Furthermore, disruption of the cell contractile machinery decreases $\alpha_5\beta_1$ -fibronectin interactions¹¹⁹. Thus, fibronectin matrix assembly is mechanically coupled to fibronectin- $\alpha_5\beta_1$ interactions.

Fibronectin matrix assembly is a stepwise process consisting of an initiation and a growth phase. During the initiation phase, fibronectin molecules bind to integrins and accumulate on the cell surface. The molecular interaction dominating this process is the binding of RGD to $\alpha_5\beta_1$, which then transmits the mechanical forces generated by the actin cytoskeleton to the fibronectin, causing conformational changes and exposing self-association sites that allow fibril elongation and maturation to proceed¹³⁴. The primary self-association site on fibronectin is the N-terminal Hep1 domain, consisting of modules I₁-I₅, as constructs without it fail to incorporate into the matrix¹³⁵. In

1 soluble fibronectin, long-range interactions between I₁-I₅ and other regions of fibronectin, including
2 III₁-III₇, mask the self-association sites protecting the molecule from aggregation. However, upon
3 integrin pulling, conformational changes propagate throughout the molecule, unmask the self-
4 association sites, activate the I₁-I₅ assembly domain and allow for matrix assembly¹³⁶. In fact,
5 addition of soluble Hep1 domain in cell culture can be incorporated into the matrix and can inhibit
6 fibronectin binding and consequently matrix assembly^{137,138}. Another feature of the fibronectin
7 molecule that is absolutely necessary for fibril formation is its dimeric character: the C-terminal
8 peptide containing the disulfide bridges. Minimal fibronectin constructs containing I₁-I₅ and the C-
9 terminal peptide (termed deminectins) could form fibrils or incorporate into an existing fibronectin
10 matrix only as disulfide-bonded dimers^{135,139,140}. The two phases of fibronectin matrix assembly can
11 be followed both biochemically and microscopically¹⁴¹. During the initiation phase of matrix
12 assembly, fibronectin binds reversibly to the cell surface and forms a molecular pool (pool I) from
13 which fibrils will form at the growth stage. The molecules in this pool are soluble to the detergent
14 deoxycholate, their electrophoretic mobility is indicative of single fibronectin molecules and at the
15 light microscopic level, they appear mainly as a punctate pattern with some very fine fibrils. Pool I of
16 cell surface-adsorbed fibronectin is then slowly transformed into a deoxycholate-insoluble matrix
17 (pool II), containing well-resolved fibrils at the light microscopic level. The fibronectin molecules in
18 pool II bind irreversibly and their electrophoretic mobility indicates a significant degree of
19 aggregation¹⁴¹.

20 Besides I₁-I₅ and the C-terminal disulfide bridges, several other regions of fibronectin have been
21 involved in fibrillogenesis, although they are not essential, in the sense that constructs lacking them
22 but containing the I₁-I₅, can still form fibrils¹³⁵. These regions include III₁-III₂¹⁴²⁻¹⁴⁴, III₄-III₈¹⁴³, and
23 III₁₂-III₁₄^{143,145}. The involvement or requirement of these domains for matrix assembly has been
24 ascertained by using constructs lacking them or by blocking antibodies. However, the exact
25 interactions between fibronectin molecules within the fibers of the extracellular matrix are not
26 known. Although elements of periodicity have been reported in fibronectin fibrils, which are

consistent with a model of staggered fibronectin molecules arranged in an antiparallel fashion, the heterogeneity of the fiber population makes it difficult to identify the exact self-association sites¹⁴⁶⁻¹⁵⁰. A recent *in silico* study¹⁵¹ suggests that the existence of multiple binding sites within the type III modules that can participate in self-assembling interactions between fibronectin molecules results in more robust fibrillogenesis, with a heterogeneous fibril population, including small and large fibers, that better resembles the cell-derived fibronectin matrix. Interestingly, the exact position of these sites within fibronectin and the mechanical and chemical properties of their constituent modules has very little influence in the resulting fibrillogenesis.

Heparin and matrix assembly

A recent study showed that fibronectin matrix assembly does not proceed in the absence of heparan sulfate proteoglycans on the cell surface¹⁵². The ability of heparan sulfate chains to bind multiple fibronectin molecules through the Hep2 domain seems to be a prerequisite for fibronectin fibrillogenesis. These observations, taken together with the catalytic effect of heparin/heparan sulfate on fibronectin conformation, suggest that besides the exposure of cryptic growth factor binding sites, heparin/heparan sulfate may also expose self-association sites necessary for fibrillogenesis. The first evidence for such an effect came from early studies of plasma cryoprecipitation¹⁵³. It was observed that heparin promotes the precipitation of fibronectin, especially at low temperatures, leading to the formation of fibrillar material¹⁵⁴. Fluorescence polarization studies¹⁵⁵ showed that a conformational change exposing self-association sites, and leading to protein aggregation and ultimately to precipitation, takes place following weak but fast interactions between fibronectin and heparin in solution.

Interestingly, all fibronectin self-association sites required for fibrillogenesis have heparin-binding affinity, suggesting the intriguing hypothesis that the classical integrin-mediated fibrillogenesis pathways may be linked in some level with interactions with heparin and/or heparan sulfate proteoglycans. Indeed, a strong correlation between the heparin-binding capacity of the Hep2

1 domain of fibronectin and its ability to mediate or modulate cell adhesion has been repeatedly
2 demonstrated for a large number of healthy and cancer cell types, including fibroblasts^{67,156-161},
3 platelets¹⁶², neural¹⁶³, endothelial^{164,165}, kidney¹⁵⁹, corneal¹⁶⁶, mammary epithelial¹⁶⁷ and bone-
4 derived¹⁶⁸ cells, as well as melanoma^{67,169-172}, neuroblastoma^{173,174}, lung carcinoma^{67,175},
5 fibrosarcoma¹⁷⁴, renal carcinoma¹⁷⁴, squamous cell carcinoma¹⁷⁶, osteosarcoma¹⁵⁹, and colon
6 carcinoma¹⁷⁷ cells. The mechanisms mediating cell adhesion and the observed phenotype are highly
7 dependent on the cell type, highlighting the complexity of the molecular interactions involving the
8 Hep2 domain. For melanoma cells¹⁶⁹, neural cells^{163,173} and endothelial cells¹⁶⁴, Hep2 alone is
9 sufficient to mediate cell adhesion, acting independently of the RGD domain. In the course of these
10 studies, it was found that Hep2 contains multiple adhesion sites (at least 7 distinct sites), some of
11 which recognize the heparan sulfate chains of proteoglycans on the cell surface, whereas others bind
12 to integrins different than $\alpha_5\beta_1$ (RGD-independent adhesion), and most frequently $\alpha_4\beta_1$ ^{170,173,178,179}.
13 It is possible that contiguous heparin- and integrin-binding sites on Hep2 bring the two adhesive
14 complexes together as a necessary step for cell adhesion¹⁶³. However, in most cases, Hep2 acts
15 cooperatively with the RGD adhesion site. A very frequent observation is that cells may attach to
16 isolated RGD or Hep2, but they require both domains to fully spread and develop focal adhesions and
17 stress fibers. This depends either on proteoglycan binding or on integrin binding to
18 Hep2^{158,160,166,171,175,177}. It is interesting to note that addition of one domain (or even an isolated
19 peptide derived from each domain) in solution is sufficient to rescue the adhesive phenotype,
20 suggesting that the key feature is the co-activation of the two complexes and not their spatial
21 distribution. However, minimal fusion constructs containing the two domains, such as III₈-III₁₀+III₁₂-
22 III₁₄¹⁷⁴ and III₇-III₁₀+III₁₃¹⁵⁹, often work as well as full-length fibronectin, whereas adsorption of
23 domain mixtures does not. This speaks for the theory that requires a certain spatial orientation of
24 the two adhesive complexes and possibly an interaction between them in order to gain full function.
25 Moreover, as already mentioned above, the interactions with the heparin-binding sites of Hep2 seem

1 to be conformation sensitive: several Hep2-derived peptides with heparin-binding capacity require
2 conjugation to a large carrier protein to exhibit full functionality^{157,163}.

3 These multiple adhesion mechanisms to one extracellular matrix protein may have a redundant
4 role, increasing the robustness of the cell response, or they may have different functions, for example,
5 fine-tuning the balance between adhesion (through $\alpha_5\beta_1$) and migration (through $\alpha_4\beta_1$). However,
6 the possibility that interaction of fibronectin with the heparan sulfate chains of the cell-surface
7 proteoglycans may be a necessary step to expose self-association sites necessary for fibrillogenesis
8 to occur suggests novel regulatory roles for proteoglycans on sites of adhesion and fibrillogenesis.
9 Future studies can determine whether this happens in concert with the well-documented RGD-
10 mediated mechanical unfolding of fibronectin that initiates fibrillogenesis or even required for it.

11

12 Fibronectin supramolecular assemblies and amyloids

13 The ability to undergo fibrillogenesis is one of the most prominent features of fibronectin. The majority
14 of fibronectin functions depends on its assembly into a fibrillar matrix through a complex cell-mediated
15 process¹²¹. However, several physical and chemical factors can promote the formation of fibrillar
16 fibronectin structures in the absence of cells, as summarized in Figure 3A. A common element between
17 these diverse mechanisms is the exposure of self-association sites, many of which are cryptic in order to
18 keep the molecule soluble, and they are exposed only upon appropriate stimulation. This can occur by
19 mechanical unfolding^{180–182}, surface adsorption^{183–185}, denaturation¹⁸⁶ or proteolysis¹⁸⁷ (the site can be
20 exposed in a fragment but remain masked in the context of the full-length molecule). Several type III
21 modules, including III_{12–14} and III₁, possess cryptic binding sites for the N-terminal part of fibronectin
22 (I₁–I₅), which, as already mentioned, may be involved in fibronectin fibrillogenesis¹²¹. It is therefore possible
23 that artificial fibronectin multimerization and fibrillar assembly may illuminate certain aspects of cell-
24 driven fibrillogenesis and matrix assembly.

1

2 **Figure 3. Fibronectin fibrillogenesis and multimerization.** (A) The ability of fibronectin to undergo

3 fibrillogenesis is an inherent property of the molecule. During extracellular matrix assembly, cells drive

4 fibronectin fibrillogenesis by using a combination of biochemical fibronectin-integrin interactions and

5 traction forces⁴⁴. However, the formation of fibrillar structures and networks can be promoted artificially,

6 by chemical or physical forces that can expose self-association sites on fibronectin molecules. Several of

7 such factors, including shear forces⁴⁵⁻⁴⁷, surface adsorption^{48,49}, chemical denaturation⁵¹, and interactions

8 with polyanions²⁰⁹, heparin¹⁸² and peptides such as anastellin⁷⁹ are listed, along with the fibrillar material

9 produced in each case. (B) High-resolution NMR structure of a complex between anastellin (blue) and

10 module III₁ of fibronectin (red), demonstrating the β -swapping mechanism: two β -strands from III₁ are

11 unfolded and substituted with the two β -strands from anastellin. (C) Schematic representation of a

12 hypothetical β -swapping mechanism that could support fibronectin fibrillogenesis. Type III modules from

13 different fibronectin molecules (each shown in a different color) interact with each other through a β -

1 swapping mechanism, with each module offering one β -strand. The connection of this β -strand with the
2 rest of the module is shown as a dashed line, drawn intentionally larger for clarity.

3

4 Cryptic self-association sites in III₁ are largely responsible for fibronectin polymerization in the absence
5 of cells into a matrix termed super-fibronectin, which is related but functionally distinct from cell-derived
6 fibrillar fibronectin¹⁸⁸. However, it has been proposed that super-fibronectin can restore the normal
7 fibronectin matrix, which is lost upon oncogenic transformation, as *ex vivo* treatment of tumor cells
8 with super-fibronectin or intraperitoneal administration of it inhibits tumor metastasis¹⁸⁹. These
9 observations, suggesting similarities between cell-derived fibronectin fibrillogenesis and super-
10 fibronectin formation, prompted intensive studies to unravel the mechanism of super-fibronectin
11 formation. Consequently, a C-terminal peptide from III₁ was identified, which was sufficient to induce
12 super-fibronectin formation and possessed anti-angiogenic, anti-metastatic and anti-tumorigenic
13 properties when administered systemically¹⁹⁰. Based on these properties, the peptide was termed
14 anastellin (from the Greek '*anastello*', meaning 'to inhibit').

15 These functions of anastellin depend upon its ability to interact with fibronectin¹⁹¹. The binding
16 sites of anastellin on fibronectin have been localized on modules III₁-III₃ and III₁₁, with modules III₁-
17 III₃ being the primary binding site, able to interact with 3 anastellin molecules¹⁹². Solution NMR
18 studies of III₃ showed that it has low chemical stability and a high unfolding rate constant compared
19 to other type III modules, suggesting its propensity to open up and expose β -strands that could
20 interact with anastellin¹⁹³. Indeed, the solution NMR structure of a complex between III₃ and
21 anastellin showed two of the β -strands of III₃ unfolded, possibly exposing further self-association
22 sites, and substituted in the complex by the two β -strands of anastellin¹⁹⁴ (Figure 3B).

23 Anastellin can also induce super-fibronectin-like aggregates when interacting with the isolated
24 domain III₁-III₂¹⁹⁵. For this to occur, the instability and consequent unfolding of III₂ are essential.
25 These observations support a mechanism whereby spontaneous unfolding of the target sequences
26 allows anastellin binding and exposure of further self-association sites leading to fibrillogenesis.

1 Interestingly, I₁-I₉ can be incorporated to the III₁-III₂/anastellin aggregates, suggesting that
2 fibronectin fibrillogenesis *in vivo* (which depends on self-association sites on I₁-I₅) may follow a
3 similar mechanism with that underlying anastellin-induced fibrillogenesis. This can happen through
4 a β -strand swapping mechanism involving interactions between type III modules, where unfolding
5 of β -strands from one module offers binding sites for β -strands from neighboring modules (Figure
6 3C). Although aggregation of the full-length III₁ module does not occur spontaneously, inner β -
7 strands can become exposed upon partial denaturation, proteolysis, mutations or mechanical stress,
8 leading to fibrillogenesis in an anastellin-like manner¹⁹⁶. However, additional mechanisms may be
9 involved, such as the formation of a tandem β -zipper between consecutive type III modules and I₁-I₅,
10 similar to the one observed upon binding of bacterial adhesins on I₁-I₅¹⁹⁷.

11 Certain structural features of anastellin resemble those of amyloid precursors, namely the lack of
12 β -strands normally found in the intact module, leading to the exposure of an extensive hydrophobic
13 surface able to mediate aggregation¹⁹⁶. This raises the exciting possibility that some aspects of
14 fibronectin fibrillogenesis *in vivo* may resemble amyloid fibril formation, following not the classical
15 β -sheet helix formation but through β -strand swapping. Further support for this hypothesis comes
16 from studies of III₉, another type III module with low chemical stability, which can undergo
17 spontaneous polymerization under physiological conditions through an inherently unstable,
18 partially unfolded intermediate, which exposes self-association sites¹⁹⁸. The resulting fibrils exhibit
19 amyloid-like properties, and it was suggested that they are formed through β -strand swapping¹⁹⁸.
20 Fibronectin fibrillogenesis based on the β -strand swapping mechanism has also been induced by a
21 III₁₀-derived peptide, which could become exposed upon integrin-mediated pulling on fibronectin in
22 physiological conditions¹⁹⁹.

23 The idea that fibronectin can form amyloid-like fibrils merits further investigation for both its
24 physiological functions (matrix assembly and aggregation during blood clotting, wound healing and
25 tissue regeneration), as well as potential pathological repercussions (abnormal depositions in
26 fibrotic diseases and lesions). Recently, it was shown that plasma fibronectin can form amyloid-like

1 aggregates upon thermal unfolding, which exhibit impaired binding capacity for gelatin and RGD
2 peptides²⁰⁰, but they are not cytotoxic and can regulate fibronectin matrix assembly, cell morphology
3 and motility, and integrin distribution in healthy and cancer cells²⁰¹.

4 The formation of amyloid-like β -sheet supramolecular structures during fibronectin multimerization is
5 also supported by the crystallographic study of the collagen-binding domain of fibronectin in the presence
6 of Zn^{2+} , already discussed earlier⁴⁶. In this study, a homodimer generated by a crystallographic twofold
7 symmetry axis was observed, with a very extensive interface involving the super-domain β -sheet C-
8 terminal lobe of the fragment. Remarkably, residues from both protomers contribute to the Zn^{2+} binding
9 site, suggesting that Zn^{2+} mediates dimerization. The Zn^{2+} -induced homodimerization of the collagen-
10 binding domain was also observed in solution⁴⁶, strengthening the crystallographic observations.
11 Intriguingly, Zn^{2+} promotes β -amyloid peptide ($\text{A}\beta$) aggregation, with possible implications for the
12 pathogenesis of Alzheimer's disease^{202,203}. Although the mechanism of Zn^{2+} is not entirely understood, there
13 is a positive correlation between high concentrations of Zn^{2+} and $\text{A}\beta$ aggregation both *in vitro* and *in vivo*^{204–}
14 ²⁰⁷. Similar to the case of the collagen-binding domain of fibronectin, Zn^{2+} interacts with certain histidine
15 residues in $\text{A}\beta$, although the geometry of complexation is different²⁰⁸. Furthermore, in contrast to the effect
16 on fibronectin structure, Zn^{2+} binding to $\text{A}\beta$ decreases the overall content of β -sheet secondary structure
17 elements^{208,209}. Despite the differences in the underlying mechanisms, it is possible that there are similarities
18 between amyloid fibril formation and fibronectin fibrillogenesis.

19

20

21 Generation of fibronectin fibers in cell-free systems

22 Initially, fibronectin was considered perhaps the only protein of the extracellular matrix unable to
23 undergo fibrillogenesis in the absence of cells²¹⁰. However, as already discussed, many different
24 chemical agents, such as heparin¹⁵⁴, polyamines²¹¹, guanidinium hydrochloride¹⁸⁶ and anastellin¹⁹⁶
25 could induce the formation of fibrillar fibronectin structures *in vitro*. In some of these cases, as for
26 example in the presence of anastellin, fiber formation proceeds through module unfolding and β -
27 strand swapping¹⁹⁴. Additionally, mechanical forces or adsorption on surfaces of appropriate

1 chemistry can lead to the generation of fibronectin fibrillar networks, as summarized in Figure 3A.
2 These observations, together with our current understanding of how integrins pull at fibronectin
3 molecules accumulated on the cell surface and expose self-association sites in order to drive
4 fibrillogenesis, lead to the conclusion that chemical or mechanical stimuli able to partially unfold
5 fibronectin and expose self-association sites may lead to fibronectin multimerization and fiber
6 formation. Certain fibril features are intimately linked with the production method; however, several
7 of these ‘artificially’ produced fibronectin fibers share important aspects of geometry and molecular
8 architecture with cell-derived fibronectin fibers, and they support cell adhesion and migration. Thus,
9 they can be used both as systems for the study of fibronectin fibrillogenesis, and as basic materials
10 for the generation of tissue engineering scaffolds. Here, we will discuss some of these cell-free
11 systems of fibronectin fiber formation and their potential applications.

12 The medicinal use of superfibronectin, the anastellin-induced fibrillar fibronectin matrix, has
13 already been discussed in the previous section. A completely different way to produce fibrillar
14 fibronectin networks is through adsorption on surfaces of well-defined chemistry, a process termed
15 material-driven assembly²¹². Changes in the substrate chemistry as small as one carbon atom can
16 abolish their ability to support fibril assembly: fibronectin spontaneously organizes into a fibrillar
17 network on poly(ethyl acrylate) (PEA), whereas it remains globular on poly(methyl acrylate) (PMA).
18 Interestingly, material driven-assembly on PEA is blocked by the addition of the I₅-I₉ N-terminal
19 fragment of fibronectin, suggesting that it relies on similar self-association sites as cell-derived
20 fibronectin matrices. Moreover, the fibronectin network developed on PEA supports the binding of
21 growth factors on the Hep2 domain and enhances their activity. Implants coated with PEA,
22 fibronectin and BMP-2 showed superior healing capacity in an *in vivo* bone repair model, and
23 required much less amount of BMP-2 than traditional growth factor delivery systems²¹³. Similarly,
24 the ability of VEGF to induce vascularization was enhanced in PEA implants coated with
25 fibronectin²¹⁴. In both cases, the results depended on the ability of fibronectin fibrillar networks

1 grown on PEA to mediate simultaneous binding of integrins and soluble growth factors in a manner
2 that allows their synergistic action.

3 In a related process, termed surface-initiated fibronectin assembly or patterning on topography
4 (PoT)^{183,215}, fibronectin is adsorbed onto a topographically patterned hydrophobic
5 polydimethylsiloxane (PDMS) surface, which partially unfolds the molecule, exposing self-
6 association sites. The resulting fibronectin layer is then transferred into a thermally responsive
7 poly(N-isopropylacrylamide) (PIPAAm) surface through microcontact printing. During the final step,
8 PIPAAm is dissolved by adjusting the temperature, releasing the fibronectin layer, which has adopted
9 the pattern imprinted on the PDMS surface. Although this is not a spontaneous fibrillogenesis
10 process, it allows the formation of free-standing fibronectin nanotextiles, as complex as the pattern
11 printed onto the PDMS surface, and it can be applied to many other extracellular matrix proteins²¹⁵.
12 Fibronectin nanofabrics have been used to investigate the effects of extracellular matrix geometry
13 on cell behavior²¹⁶. Cells can even be cultured on the nanostructures before the thermally triggered
14 PIPAAm dissolution, leading to their encapsulation in the resulting nanofabric through a shrink-
15 wrapping process, without affecting their viability²¹⁷. Cell encapsulation in such nano-scaffolds can
16 be used for cell delivery applications.

17 Another major factor leading to the formation of fibronectin fibers, also employed by cells, is the
18 application of mechanical forces. Mats composed of oriented fibers can be formed upon the
19 application of shear forces on concentrated fibronectin solutions^{180,218}, and the process can be scaled-
20 up by wet extrusion in an acidic coagulation bath to produce materials that can be used as surgical
21 grafts²¹⁹. Freeze-dried and rehydrated fibronectin mats have been successfully used *in vivo* to repair
22 injuries in the peripheral and central nervous system^{220–224}. One of the essential elements in these
23 grafts is their oriented fibers, which can act as conduits for cell migration, facilitating tissue
24 regeneration and reducing scarring. This is especially important for the repair of tissues such as
25 nerve, tendon and blood vessels²²⁵.

1 Early studies showed that fibers of well-controlled orientation could be produced by pulling with
2 a rod from a fibronectin solution^{180,218}. Studies from one of the authors have shown that in this model
3 system of fibronectin fibrillogenesis, fibers are formed by pulling the fibronectin monolayer that has
4 formed at the air-liquid interface of a droplet (Figure 4)¹⁸¹. Fibronectin is known to form stable,
5 insoluble monolayers at air-liquid interfaces^{226,227}. Once the surface tension is overcome by the
6 pulling force, the fibronectin monolayer at the interface undergoes a sheet-to-fiber transition. The
7 length of the newly formed fiber increases by the continuous transfer of material from the droplet
8 surface. Sufficiently fast fibronectin adsorption from the droplet solution to the interface refurbishes
9 the material loss, and such fibers can reach lengths up to the cm range¹⁸¹. The forces required for the
10 sheet-to-fiber transition are in the mN range²²⁸. A related phenomenon is the drawing of lipid tubules
11 from phospholipid vesicles²²⁹. However, the lipid tubules retract immediately if they are not
12 connected to a vesicle at both ends²³⁰, whereas the pulled fibronectin fibers remain stable even after
13 they are cut from the fibronectin droplet. This indicates that the transition is irreversible, and the
14 resulting fiber forms a stable structure. It is likely that additionally to the sheet-to-fiber transition,
15 transformations within the fibronectin monolayer, possibly through shear-induced self-association
16 of fibronectin molecules, further stabilize the resulting fiber. As the pulling continues, the sheet
17 buckles at several points and eventually collapses into lamellar structures that curve away from the
18 centripetal direction and form topologically segregated spiral segments extending throughout the
19 length of the fiber. It should be noted that other proteins with the ability to undergo fibrillogenesis
20 and to form insoluble monolayers at the air-water interface, such as BSA, laminin and collagen I, did
21 form fibers by pulling at the interface, suggesting that specific properties of the fibronectin molecules
22 are necessary for the sheet-to-fiber transition to occur¹⁸¹. Force-induced fiber formation from a
23 fibronectin monolayer forming at the air-water interface has also been reported in a different setting,
24 using an array of micropillars, which upon wetting, bend and pull on a fibronectin solution suspended
25 between them¹⁸². In this study, a network of nanofibrils was formed, suspended between the
26 micropillars. Finally, an

Figure 4. Assembly of fibronectin fibers by pulling at the air-water interface. A schematic representation of the irreversible sheet-to-fiber phase transition. Fibronectin adsorbs at the air-water interface forming an insoluble monolayer (A). The tension of pulling and the concomitant buffer drainage induces inward folds in the fibronectin monolayer, which collapse into bilayer folds (B). The increasing space confinement during fiber growth eventually leads to the spiraling of the bilayer folds, generating topologically segregated spiral domains form, extending throughout the length of the fiber (C). Such domains are clearly visible in a transmission electron microscopy image of a fiber cross-section (D)¹⁸¹ (Reprinted from Biomaterials, 36, Maria Mitsi, Stephan Handschin, Isabel Gerber, Ruth Schwartländer, Enrico Klotzsch, Roger Wepf, Viola Vogel, The ultrastructure of fibronectin fibers pulled from a protein monolayer at the air-liquid interface and the mechanism of the sheet-to-fiber transition, 66-79, 2015, with permission from Elsevier).

additional analogy can be drawn with amyloid fibrils, again, which have been shown to form at the air-water interface by lateral aggregation²³¹.

Such manually pulled fibronectin fibers from the air-water interface bear conformational similarities with cell-derived fibronectin fibers, as has been assessed by Fluorescence-Energy Transfer (FRET)-based observations²³². Moreover, oriented fibronectin fibers of similar dimensions have been observed to colocalize with growing blood vessels in the embryo²³³. Accordingly, studies

1 from one of the authors have used such fibers to develop a model system to study the interplay
2 between fibronectin and growth factors during the early steps of neovascularization²³³. However,
3 they are not free-standing, and their production cannot easily be scaled up, which makes them more
4 suitable for research and diagnostic assays rather than tissue engineering and therapeutic
5 applications.

7 Conclusions and Future Perspectives

8 Decades of intensive research have produced a great number of pieces for the puzzle of fibronectin
9 structure. The picture that has emerged shows a complex structure-function relationship. A
10 hierarchically modular architecture with a large number of self-association sites allows fibronectin
11 to adopt many different conformations, depending on the constantly changing biomechanical
12 properties of the cellular microenvironment. Each conformation may be associated with a set of
13 different binding sites for adhesive receptors and signaling molecules, and therefore, with a different
14 functionality. At the same time, distinct conformations may keep fibronectin in solution or trigger its
15 fibrillogenesis. Fibrillar fibronectin is also amenable to conformational changes, contributing to the
16 dynamic character of the extracellular matrix. Binding sites may be formed or destroyed depending
17 on the tension applied to the matrix or the presence of structural catalysts, such as heparin. Thus, the
18 conformational flexibility of fibronectin grants it the ability to integrate signals, regulate their
19 presentation to the cells and to some extent, control the cell response. Our knowledge of the
20 underlying mechanisms is fragmented, focusing on single pathways and individual binding events.
21 Future research will help us to understand better how the different mechanisms work together, and
22 build a more comprehensive picture of the structure-function relationship.

24 FIGURE LEGENDS

25 Figure 1. The multimodular architecture of fibronectin. Schematic representation of modules type I
26 (purple), type II (green) and type III (red) constituting one chain of fibronectin. The alternatively spliced

1 type III modules EDA and EDB, as well as the alternatively spliced region V are shown in a lighter color.
2 The disulfide bridges linking the two chains at their C-termini in an antiparallel fashion are also shown.
3 Several modules are forming functional domains displaying distinct binding affinities, and they can be
4 released as stable fragments by proteolysis. The major proteolytic sites and the respective proteases are
5 shown, together with the molecular weights of the resulting fragments (bottom), and some of their binding
6 affinities (top).

7

8 Figure 2. The catalytic mechanism of heparin altering fibronectin conformation. Interactions between
9 heparin (wavy line) and fibronectin induce a conformational change from a compact (gray) to an extended
10 (red) configuration, which reduces the affinity for heparin, allowing for its dissociation, diffusion and
11 binding to neighboring fibronectin molecules. In this way, substoichiometric amounts of heparin can alter
12 the conformation of the entire fibronectin matrix, acting as a structural catalyst. Three catalytic cycles are
13 shown. One of the functional consequences of the heparin-catalyzed conformational change on fibronectin
14 is the exposure of cryptic growth factor binding sites on the Hep2 (III₁₂-III₁₄) domain of fibronectin⁶⁶.

15

16 Figure 3. Fibronectin fibrillogenesis and multimerization. (A) The ability of fibronectin to undergo
17 fibrillogenesis is an inherent property of the molecule. During extracellular matrix assembly, cells drive
18 fibronectin fibrillogenesis by using a combination of biochemical fibronectin-integrin interactions and
19 traction forces⁴⁴. However, the formation of fibrillar structures and networks can be promoted artificially,
20 by chemical or physical forces that can expose self-association sites on fibronectin molecules. Several of
21 such factors, including shear forces⁴⁵⁻⁴⁷, surface adsorption^{48,49}, chemical denaturation⁵¹, and interactions
22 with polyanions²⁰⁹, heparin¹⁸² and peptides such as anastellin⁷⁹ are listed, along with the fibrillar material
23 produced in each case. (B) High-resolution NMR structure of a complex between anastellin (blue) and
24 module III₁ of fibronectin (red), demonstrating the β -swapping mechanism: two β -strands from III₁ are
25 unfolded and substituted with the two β -strands from anastellin. (C) Schematic representation of a

hypothetical β -swapping mechanism that could support fibronectin fibrillogenesis. Type III modules from different fibronectin molecules (each shown in a different color) interact with each other through a β -swapping mechanism, with each module offering one β -strand. The connection of this β -strand with the rest of the module is shown as a dashed line, drawn intentionally larger for clarity.

Figure 4. Assembly of fibronectin fibers by pulling at the air-water interface. A schematic representation of the irreversible sheet-to-fiber phase transition. Fibronectin adsorbs at the air-water interface forming an insoluble monolayer (A). The tension of pulling and the concomitant buffer drainage induces inward folds in the fibronectin monolayer, which collapse into bilayer folds (B). The increasing space confinement during fiber growth eventually leads to the spiraling of the bilayer folds, generating topologically segregated spiral domains form, extending throughout the length of the fiber (C). Such domains are clearly visible in a transmission electron microscopy image of a fiber cross-section (D)¹⁸¹. (Reprinted from Biomaterials, 36, Maria Mitsi, Stephan Handschin, Isabel Gerber, Ruth Schwartländer, Enrico Klotzsch, Roger Wepf, Viola Vogel, The ultrastructure of fibronectin fibers pulled from a protein monolayer at the air-liquid interface and the mechanism of the sheet-to-fiber transition, 66-79, 2015, with permission from Elsevier).

AUTHOR INFORMATION

Co-corresponding authors: *Raffaele Mezzenga, *Maria Mitsi

Laboratory of Food and Soft Materials,

ETH Zurich, Switzerland

raffaele.mezzenga@hest.ethz.ch

maria.mitsi@hest.ethz.ch

REFERENCES

- (1) Zollinger, A. J.; Smith, M. L. Fibronectin, the Extracellular Glue. *Matrix Biol.* **2016**, <http://dx.doi.org/10.1016/j.matbio.2016.07.011>.
- (2) Oh, E.; Pierschbacher, M.; Ruoslahti, E. Deposition of Plasma Fibronectin in Tissues. *Proc. Natl. Acad. Sci. U. S. A.* **1981**, 78 (5), 3218–3221.
- (3) Mosher, D. F. Physiology of Fibronectin. *Annu Rev Med* **1984**, 35, 561–575.
- (4) Cho, J.; Mosher, D. F. Role of Fibronectin Assembly in Platelet Thrombus Formation. *J. Thromb. Haemost.* **2006**, 4, 1461–1469.
- (5) Czop, J. K. Phagocytosis of Particulate Activators of the Alternative Complement Pathway: Effects of Fibronectin. *Adv Immunol* **1986**, 38 (1965), 361–398.
- (6) Grinnell, F. Fibronectin and Wound Healing. *J Cell Biochem* **1984**, 26 (2), 107–116.
- (7) Astrof, S.; Hynes, R. O. Fibronectins in Vascular Morphogenesis. *Angiogenesis* **2009**, 12 (2), 165–175.
- (8) Davidson, L. A.; Dzamba, B. J.; Keller, R.; DeSimone, D. W. Live Imaging of Cell Protrusive Activity, and Extracellular Matrix Assembly and Remodeling during Morphogenesis in the Frog, *Xenopus Laevis*. *Dev. Dyn.* **2008**, 273 (2), 2684–2692.
- (9) George, E. L.; Georges-Labouesse, E. N.; Patel-King, R. S.; Rayburn, H.; Hynes, R. O. Defects in Mesoderm, Neural Tube and Vascular Development in Mouse Embryos Lacking Fibronectin. *Development* **1993**, 119 (4), 1079–1091.
- (10) George, E. L.; Baldwin, H. S.; Hynes, R. O. Fibronectins Are Essential for Heart and Blood Vessel Morphogenesis but Are Dispensable for Initial Specification of Precursor Cells. *Blood* **1997**, 90 (8), 3073–3081.
- (11) Walraven, M.; Hinz, B. Therapeutic Approaches to Control Tissue Repair and Fibrosis: Extracellular

Matrix as a Game Changer. *Matrix Biol.* **2018**, No. 2017.

(12) Stoppacciaro, A.; Pietrucci, A.; Fofi, C.; Raffa, S.; Torrisi, M. R.; Men, P. Fibronectin Glomerulopathy : An Uncommon Cause of Nephrotic Syndrome in Systemic Lupus Erythematosus. *NDT Plus* **2008**, No. 4, 225–227.

(13) Kawahara, E.; Shiroo, M.; Nakanishi, I.; Migita, S. The Role of Fibronectin in the Development of Experimental Amyloidosis. Evidence of Immunohistochemical Codistribution and Binding Property with Serum Amyloid Protein A. *Am J Pathol* **1989**, 134 (6), 1305–1314.

(14) Akiyama, S. K.; Olden, K.; Yamada, K. M. Fibronectin and Integrins in Invasion and Metastasis. *Cancer Metastasis Rev.* **1995**, 14 (3), 173–189.

(15) Morrison, P. R.; Edsall, J. T.; Miller, S. G. Preparation and Properties of Serum and Plasma Proteins. XVIII. The Separation of Purified Fibrinogen from Fraction I of Human Plasma. *J. Am. Chem. Soc.* **1948**, 70 (9), 3103–3108.

(16) Mosesson, M. W.; Umfleet, R. A. The Cold-Insoluble Globulin of Human Plasma. *J. Biol. Chem.* **1970**, 245 (21), 5728–5736.

(17) Molnar, J.; McLain, S.; Allen, C.; Laga, H.; Gara, A.; Gelder, F. The Role of an alpha2-Macroglobulin of Rat Serum in the Phagocytosis of Colloidal Particles. *Biochim. Biophys. Acta* **1977**, 493, 37–54.

(18) Wartiovaara, J.; Linder, E.; Ruoslahti, E.; Vaheri, A. Distribution of Fibroblast Surface Antigen: Association with Fibrillar Structures of Normal Cells and Loss Upon Viral Transformation. *J. Exp. Med.* **1974**, 140, 1522–1533.

(19) Chen, L. B.; Murray, A.; Segal, R. A.; Bushnell, A.; Walsh, M. L. Studies on Intercellular LETS Glycoprotein Matrices. *Cell* **1978**, 14 (2), 377–391.

(20) Vaheri, A.; Mosher, D. F. High Molecular Weight, Cell Surface-Associated Glycoprotein

(Fibronectin) Lost in Malignant Transformation. *Biochim Biophys Acta* **1978**, 516 (1), 1–25.

(21) McDonagh, J. *Plasma Fibronectin: Structure and Function*; 1985.

(22) Kar, L.; Lai, C. S.; Wolff, C. E.; Nettesheim, D.; Sherman, S.; Johnson, M. E. H-1 NMR-Based Determination of the 3-Dimensional Structure of the Human Plasma Fibronectin Fragment Containing Inter-Chain Disulfide Bonds. *J. Biol. Chem.* **1993**, 268 (12), 8580–8589.

(23) Johansson, S.; Svineng, G.; Wennerberg, K.; Armulik, A.; Lohikangas, L. Fibronectin-Integrin Interactions. *Front. Biosci.* **1997**, 2 (11), d126-46.

(24) Balian, G.; Click, E. M.; Bornstein, P. Location of a Collagen-Binding Domain in Fibronectin. *J Biol Chem* **1980**, 255 (8), 3234–3236.

(25) Yamada, K. M.; Kennedy, D. W.; Kimata, K.; Pratt, R. M. Characterization of Fibronectin Interactions with Glycosaminoglycans and Identification of Active Proteolytic Fragments. *J Biol Chem* **1980**, 255 (13), 6055–6063.

(26) Corbett, S. A.; Schwarzbauer, J. E. Fibronectin-Fibrin Cross-Linking: A Regulator of Cell Behavior. *Trends Cardiovasc. Med.* **1998**, 8 (8), 357–362.

(27) Calaycay, J.; Pande, H.; Lee, T.; Borsi, L.; Siri, A.; Shively, J. E.; Zardi, L. Primary Structure of a DNA- and Heparin-Binding Domain (Domain III) in Human Plasma Fibronectin. *J Biol Chem* **1985**, 260 (22), 12136–12141.

(28) Sekiguchi, K.; Hakomori, S. Functional Domain Structure of Fibronectin. *Proc. Natl. Acad. Sci.* **1980**, 77 (5), 2661–2665.

(29) Khan, M. Y.; Medow, M. S.; Newman, S. A. Unfolding Transitions of Fibronectin and Its Domains - Stabilization and Structural Alteration of the N-Terminal Domain by Heparin. *Biochem. J.* **1990**, 270 (1), 33–38.

(30) de Carreira, P. F.; Castellino, F. J. The Thermotropic Properties of Human Plasma Fibronectin. *Arch.*

Biochem. Biophys. **1985**, 243 (1), 284–291.

(31) Ingham, K. C.; Brew, S. A.; Broekelmann, T. J.; McDonald, J. A. Thermal Stability of Human Plasma Fibronectin and Its Constituent Domains. *J. Biol. Chem.* **1984**, 259 (19), 11901–11907.

(32) Tatunashvili, L. V.; Filimonov, V. V.; Privalov, P. L.; Metsis, M. L.; Koteliansky, V. E.; Ingham, K. C.; Medved, L. V. Co-Operative Domains in Fibronectin. *J. Mol. Biol.* **1990**, 211 (1), 161–169.

(33) Pankov, R.; Yamada, K. M. Fibronectin at a Glance. *J Cell Sci* **2002**, 115 (Pt 20), 3861–3863.

(34) Schwarzbauer, J. E. Alternative Splicing of Fibronectin: Three Variants, Three Functions. *Bioessays* **1991**, 13 (10), 527–533.

(35) Litvinovich, S. V.; Strickland, D. K.; Ingham, K. C. Domain Structure and Interactions Modules in the Gelatin-Binding of the Type I and Type II Region of Fibronectin Folded. *J. Mol. Biol.* **1991**, 217, 563–575.

(36) Litvinovich, S. V.; Ingham, K. C. Interactions between Type III Domains in the 110 kDa Cell-Binding Fragment of Fibronectin. *J Mol Biol* **1995**, 248 (3), 611–626.

(37) Novokhatny, V.; Schwarz, F.; Atha, D.; Ingham, K. Domain Structure and Domain-Domain Interactions in the Carboxy-Terminal Heparin Binding Region of Fibronectin. *J Mol Biol* **1992**, 227 (4), 1182–1191.

(38) Rudiño-Piñera, E.; Ravelli, R. B. G.; Sheldrick, G. M.; Nanao, M. H.; Korostelev, V. V.; Werner, J. M.; Schwarz-linek, U.; Potts, J. R.; Garman, E. F. The Solution and Crystal Structures of a Module Pair from the Staphylococcus Aureus -Binding Site of Human Fibronectin — A Tale with a Twist. *J. Mol. Biol.* **2007**, 368, 833–844.

(39) Williams, M. J.; Phan, I.; Harvey, T. S.; Rostagno, A.; Gold, L. I.; Campbell, I. D. Solution Structure of a Pair of Fibronectin Type 1 Modules with Fibrin Binding Activity. *J Mol Biol* **1994**, 235 (4), 1302–1311.

- 1 (40) Smith, S. P.; Hashimoto, Y.; Pickford, A. R.; Campbell, I. D.; Werner, M. Interface Characterization
2 of the Type II Module Pair from Fibronectin. *Biochemistry* **2000**, *39*, 8374–8381.
- 3 (41) Bocquier, A. A.; Potts, J. R.; Pickford, A. R.; Campbell, I. D. Solution Structure of a Pair of Modules
4 from the Gelatin-Binding Domain of Fibronectin. *Structure* **1999**, *7* (12), 1451–1460.
- 5 (42) Hashimoto, Y.; Smith, S. P.; Pickford, A. R.; Bocquier, A. A.; Campbell, I. D.; Werner, J. M. The
6 Relative Orientation of the Fibronectin (6)F1(1)F2 Module Pair: A N-15 NMR Relaxation Study. *J.*
7 *Biomol. Nmr* **2000**, *17* (3), 203–214.
- 8 (43) Johnson, K. J.; Sage, H.; Briscoe, G.; Erickson, H. P. The Compact Conformation of Fibronectin Is
9 Determined by Intramolecular Ionic Interactions. *J. Biol. Chem.* **1999**, *274* (22), 15473–15479.
- 10 (44) Pickford, A. R.; Smith, S. P.; Staunton, D.; Boyd, J.; Campbell, I. D. The Hairpin Structure of the
11 (6)F1(1)F2(2)F2 Fragment from Human Fibronectin Enhances Gelatin Binding. *Embo J* **2001**, *20*
12 (7), 1519–1529.
- 13 (45) Erat, M. C.; Schwarz-Linek, U.; Pickford, A. R.; Farndale, R. W.; Campbell, I. D.; Vakonakis, I.
14 Implications for Collagen Binding from the Crystallographic Structure of Fibronectin 6FnI1-
15 2FnII7FnI. *J. Biol. Chem.* **2010**, *285* (44), 33764–33770.
- 16 (46) Graille, M.; Pagano, M.; Rose, T.; Ravoux, M. R.; Van Tilbeurgh, H. Zinc Induces Structural
17 Reorganization of Gelatin Binding Domain from Human Fibronectin and Affects Collagen Binding.
18 *Structure* **2010**, *18*, 710–718.
- 19 (47) Erat, C.; Slatter, D. A.; Lowe, E. D.; Millard, C. J.; Farndale, R. W.; Campbell, I. D. Identification
20 and Structural Analysis of Type I Collagen Sites in Complex with Fibronectin Fragments. *Proc.*
21 *Natl. Acad. Sci.* **2009**, *106* (11), 4195–4200.
- 22 (48) Williams, E. C.; Janmey, P. A.; Ferry, J. D.; Mosher, D. F. Conformational States of Fibronectin.
23 Effects of pH, Ionic Strength, and Collagen Binding. *J Biol Chem* **1982**, *257* (24), 14973–14978.

- 1 (49) Erickson, H. P.; Carrell, N. A. Fibronectin in Extended and Compact Conformations. Electron
2 Microscopy and Sedimentation Analysis. *J Biol Chem* **1983**, 258 (23), 14539–14544.
- 3 (50) Bergkvist, M.; Carlsson, J.; Oscarsson, S. Surface-Dependent Conformations of Human Plasma
4 Fibronectin Adsorbed to Silica, Mica, and Hydrophobic Surfaces, Studied with Use of Atomic Force
5 Microscopy. *J Biomed Mater Res* **2003**, 64A (2), 349–356.
- 6 (51) Vogel, V. Mechanotransduction Involving Multimodular Proteins: Converting Force into
7 Biochemical Signals. *Annu Rev Biophys Biomol Struct* **2006**, 35, 459–488.
- 8 (52) Maurer, L. M.; Ma, W.; Mosher, D. F. Dynamic Structure of Plasma Fibronectin. *Crit. Rev. Biochem.*
9 *Mol. Biol.* **2016**, 51 (4), 231–227.
- 10 (53) Rocco, M.; Infusini, E.; Daga, M. G.; Gogioso, L.; Cuniberti, C. Models of Fibronectin. *Embo J.*
11 **1987**, 6 (8), 2343–2349.
- 12 (54) Iozzo, R. V. Matrix Proteoglycans: From Molecular Design to Cellular Function. *Annu Rev Biochem*
13 **1998**, 67, 609–652.
- 14 (55) Lindahl, U. “Heparin” - from Anticoagulant Drug into the New Biology. *Glycoconj J* **2000**, 17 (7–
15 9), 597–605.
- 16 (56) Metcalfe, D. D.; Baram, D.; Mekori, Y. A. Mast Cells. *Physiol. Rev.* **1997**, 77 (4), 1033–1079.
- 17 (57) Mulloy, B.; Forster, M. J. Conformation and Dynamics of Heparin and Heparan Sulfate.
18 *Glycobiology* **2000**, 10 (11), 1147–1156.
- 19 (58) Richter, H.; Seidl, M.; Hörmann, H. Location of Heparin-Binding Sites of Fibronectin. Detection of
20 a Hitherto Unrecognized Transamidase. *Hoppe Seylers Z Physiol Chem* **1981**, 362, 399–408.
- 21 (59) Gold, L. I.; Frangione, B.; Pearlstein, E. Biochemical and Immunological Characterization of Three
22 Binding Sites on Human Plasma Fibronectin with Different Affinities for Heparin. *Biochemistry*
23 **1983**, 22 (17), 4113–4119.

- 1 (60) Benecky, M. J.; Kolvenbach, C. G.; Amrani, D. L.; Mosesson, M. W. Evidence That Binding to the
2 Carboxyl-Terminal Heparin-Binding Domain (Hep II) Dominates the Interaction between Plasma
3 Fibronectin and Heparin. *Biochemistry* **1988**, 27 (19), 7565–7571.
- 4 (61) Hayashi, M.; Yamada, K. M. Divalent Cation Modulation of Fibronectin Binding to Heparin and to
5 DNA. *J. Biol. Chem.* **1982**, 257 (9), 5263–5267.
- 6 (62) Ingham, K. C.; Brew, S. A.; Athat, D. H. Interaction of Heparin with Fibronectin and Isolated
7 Fibronectin Domains. *Biochem. J.* **1990**, 272, 605–611.
- 8 (63) Kang, W.; Park, S.; Jang, J. H. Kinetic and Functional Analysis of the Heparin-Binding Domain of
9 Fibronectin. *Biotechnol. Lett.* **2008**, 30 (1), 55–59.
- 10 (64) Lin, H.; Lal, R.; Clegg, D. O. Imaging and Mapping Heparin-Binding Sites on Single Fibronectin
11 Molecules with Atomic Force Microscopy. *Biochemistry* **2000**, 39 (12), 3192–3196.
- 12 (65) Mitchell, G.; Lamontagne, C. A.; Lebel, R.; Grandbois, M.; Malouin, F. Single-Molecule Dynamic
13 Force Spectroscopy of the Fibronectin-Heparin Interaction. *Biochem. Biophys. Res. Commun.* **2007**,
14 364 (3), 595–600.
- 15 (66) Mitsi, M.; Forsten-Williams, K.; Gopalakrishnan, M.; Nugent, M. A. A Catalytic Role of Heparin
16 within the Extracellular Matrix. *J Biol Chem* **2008**, 283 (50), 34796–34807.
- 17 (67) Makabe, T.; Saiki, I.; Murata, J.; Ohdate, Y.; Kawase, Y.; Taguchi, Y.; Shimojo, T.; Kimuzuka, F.;
18 Kato, I.; Azuma, I. Modulation of Haptotactic Migration of Metastatic Melanoma Cells by the
19 Interaction between Heparin and Heparin-Binding Domain of Fibronectin. *J. Biol. Chem.* **1990**, 265
20 (24), 14270–14276.
- 21 (68) Ingham, K. C.; Brew, S. A.; Migliorini, M. M.; Busby, T. F. Binding of Heparin by Type III Domains
22 and Peptides from the Carboxy Terminal Hep-2 Region of Fibronectin. *Biochemistry* **1993**, 32 (46),
23 12548–12553.

- 1 (69) Barkalow, F. J. B.; Schwarzbauer, J. E. Localization of the Major Heparin-Binding Site in
2 Fibronectin. *J. Biol. Chem.* **1991**, 266 (12), 7812–7818.
- 3 (70) Ingham, K. C.; Brew, S. A.; Migliorini, M. An Unusual Heparin-Binding Peptide from the Carboxy-
4 Terminal Hep-2 Region of Fibronectin. *Arch. Biochem. Biophys.* **1994**, 314 (2), 242–246.
- 5 (71) Mohri, H.; Katoh, K.; Motomura, S.; Okubo, T. Novel Synthetic Peptides from the C-Terminal
6 Heparin Binding Domain of Fibronectin with Heparin Binding Activity. *Peptides* **1996**, 17 (6),
7 1079–1081.
- 8 (72) Busby, T. F.; Argraves, W. S.; Brew, S. A.; Pechik, I.; Gilliland, G. L.; Ingham, K. C. Heparin
9 Binding by Fibronectin Module III-13 Involves Six Discontinuous Basic Residues Brought Together
10 to Form a Cationic Cradle. *Journal of Biological Chemistry*. **1995**, 270 (31), 18558–18562.
- 11 (73) Ogamo, A.; Nagai, A.; Nagasawa, K. Binding of Heparin Fractions and Other Polysulfated
12 Polysaccharides to Plasma Fibronectin: Effects of Molecular Size and Degree of Sulfation of
13 Polysaccharides. *BBA - Gen. Subj.* **1985**, 841 (1), 30–41.
- 14 (74) Walker, A.; Gallagher, J. T. Structural Domains of Heparan Sulphate for Specific Recognition of the
15 C-Terminal Heparin-Binding Domain of Human Plasma Fibronectin (HEPII). *Biochem J* **1996**, 317
16 (Pt 3), 871–877.
- 17 (75) Lyon, M.; Rushton, G.; Askari, J. A.; Humphries, M. J.; Gallagher, J. T. Elucidation of the Structural
18 Features of Heparan Sulfate Important for Interaction with the Hep-2 Domain of Fibronectin. *J Biol*
19 *Chem* **2000**, 275 (7), 4599–4606.
- 20 (76) Mahalingam, Y.; Gallagher, J. T.; Couchman, J. R. Cellular Adhesion Responses to the Heparin-
21 Binding (HepII) Domain of Fibronectin Require Heparan Sulfate with Specific Properties. *J Biol*
22 *Chem* **2007**, 282 (5), 3221–3230.
- 23 (77) Mitsi, M.; Hong, Z.; Costello, C. E.; Nugent, M. A. Heparin-Mediated Conformational Changes in
24 Fibronectin Expose Vascular Endothelial Growth Factor Binding Sites. *Biochemistry* **2006**, 45 (34),

10319–10328.

(78) Hubbard, B.; Buczek-Thomas, J. A.; Nugent, M. A.; Smith, M. L. Heparin-Dependent Regulation of Fibronectin Matrix Conformation. *Matrix Biol.* **2014**, *34*, 124–131.

(79) Niedzwiadek, W. E.; O'Bryan, G. T.; Blumenstock, F. A.; Saba, T. M.; Andersen, T. T. A Calorimetric Analysis of Human Plasma Fibronectin: Effects of Heparin Binding on Domain Structure. *Biochemistry* **1988**, *27* (18), 7116–7124.

(80) Smith, E. M.; Mitsi, M.; Nugent, M. A.; Symes, K. PDGF-A Interactions with Fibronectin Reveal a Critical Role for Heparan Sulfate in Directed Cell Migration during *Xenopus* Gastrulation. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, *106* (51), 21683–21688.

(81) Martino, M. M.; Hubbell, J. A. The 12th-14th Type III Repeats of Fibronectin Function as a Highly Promiscuous Growth Factor-Binding Domain. *Faseb J* **2010**, *24* (12), 4711–4721.

(82) Jones, J. I.; Gockerman, A.; Busby, W. H. J.; Camacho-Hubner, C.; Clemmons, D. R. Extracellular Matrix Contains Insulin-like Growth Factor Binding Protein-5: Potentiation of the Effects of IGF-I. *J. Cell Biol.* **1993**, *121* (3), 679–687.

(83) Gui, Y.; Murphy, L. J. Insulin-like Growth Factor (IGF)-Binding Protein-3 (IGFBP-3) Binds to Fibronectin (FN): Demonstration of IGF-I/IGFBP-3/FN Ternary Complexes in Human Plasma. *J. Clin. Endocrinol. Metab.* **2001**, *86* (5), 2104–2110.

(84) Rahman, S.; Patel, Y.; Murray, J.; Patel, K. V; Sumathipala, R.; Sobel, M.; Wijelath, E. S. Novel Hepatocyte Growth Factor (HGF) Binding Domains on Fibronectin and Vitronectin Coordinate a Distinct and Amplified Met-Integrin Induced Signalling Pathway in Endothelial Cells. *BMC Cell Biol* **2005**, *6* (1), 8.

(85) Pi, L.; Ding, X.; Jorgensen, M.; Pan, J. J.; Oh, S. H.; Pintilie, D.; Brown, A.; Song, W. Y.; Petersen, B. E. Connective Tissue Growth Factor with a Novel Fibronectin Binding Site Promotes Cell Adhesion and Migration during Rat Oval Cell Activation. *Hepatology* **2008**, *47* (3), 996–1004.

- (86) Wijelath, E. S.; Rahman, S.; Namekata, M.; Murray, J.; Nishimura, T.; Mostafavi-Pour, Z.; Patel, Y.; Suda, Y.; Humphries, M. J.; Sobel, M. Heparin-II Domain of Fibronectin Is a Vascular Endothelial Growth Factor-Binding Domain: Enhancement of VEGF Biological Activity by a Singular Growth Factor/matrix Protein Synergism. *Circ. Res.* **2006**, *99* (8), 853–860.
- (87) Small, A. R.; Neagu, A.; Amyot, F.; Sackett, D.; Chernomordik, V.; Gandjbakhche, A. Spatial Distribution of VEGF Isoforms and Chemotactic Signals in the Vicinity of a Tumor. *J Theor Biol* **2008**, *252* (4), 593–607.
- (88) Ruhrberg, C.; Gerhardt, H.; Golding, M.; Watson, R.; Ioannidou, S.; Fujisawa, H.; Betsholtz, C.; Shima, D. T. Spatially Restricted Patterning Cues Provided by Heparin-Binding VEGF-A Control Blood Vessel Branching Morphogenesis. *Genes Dev* **2002**, *16* (20), 2684–2698.
- (89) Chen, T. T.; Luque, A.; Lee, S.; Anderson, S. M.; Segura, T.; Iruela-Arispe, M. L. Anchorage of VEGF to the Extracellular Matrix Conveys Differential Signaling Responses to Endothelial Cells. *J. Cell Biol.* **2010**, *188* (4), 595–609.
- (90) Anderson, S. M.; Chen, T. T.; Iruela-Arispe, M. L.; Segura, T. The Phosphorylation of Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2) by Engineered Surfaces with Electrostatically or Covalently Immobilized VEGF. *Biomaterials* **2009**, *30* (27), 4618–4628.
- (91) Lin, F.; Ren, X. D.; Pan, Z.; Macri, L.; Zong, W. X.; Tonnesen, M. G.; Rafailovich, M.; Bar-Sagi, D.; Clark, R. A. Fibronectin Growth Factor-Binding Domains Are Required for Fibroblast Survival. *J Invest Dermatol* **2011**, *131* (1), 84–98.
- (92) Lin, F.; Zhu, J.; Tonnesen, M. G.; Taira, B. R.; McClain, S. A.; Singer, A. J.; Clark, R. A. F. Fibronectin Peptides That Bind PDGF-BB Enhance Survival of Cells and Tissue under Stress. *J Invest Dermatol* **2014**, *134* (4), 1119–1127.
- (93) Sawicka, K. M.; Seeliger, M.; Musaev, T.; Macri, L. K.; Clark, R. A. F. Fibronectin Interaction and Enhancement of Growth Factors : Importance for Wound Healing. *Adv. Wound Care* **2015**, *4* (8),

469–478.

- (94) Chabria, M.; Hertig, S.; Smith, M. L.; Vogel, V. Stretching Fibronectin Fibres Disrupts Binding of Bacterial Adhesins by Physically Destroying an Epitope. *Nat Commun* **2010**, *1*, 135.
- (95) Kubow, K. E.; Vukmirovic, R.; Zhe, L.; Klotzsch, E.; Smith, M. L.; Gourdon, D.; Luna, S.; Vogel, V. Mechanical Forces Regulate the Interactions of Fibronectin and Collagen I in Extracellular Matrix. *Nat. Commun.* **2015**, *6*, 8026.
- (96) Little, W. C.; Schwartlander, R.; Smith, M. L.; Gourdon, D.; Vogel, V. Stretched Extracellular Matrix Proteins Turn Fouling and Are Functionally Rescued by the Chaperones Albumin and Casein. *Nano Lett* **2009**, *9* (12), 4158–4167.
- (97) Ortiz Franyuti, D.; Mitsi, M.; Vogel, V. Mechanical Stretching of Fibronectin Fibers Upregulates Binding of Interleukin-7. *Nano Lett.* **2018**, *18* (1), 15–25.
- (98) Hynes, R. O. Integrins: Versatility, Modulation, and Signaling in Cell Adhesion. *Cell* **1992**, *69* (1), 11–25.
- (99) Baron, M.; Main, A. L.; Driscoll, P. C.; Mardon, H. J.; Boyd, J.; Campbell, I. D. ¹H NMR Assignment and Secondary Structure of the Cell Adhesion Type III Module of Fibronectin. *Biochemistry* **1992**, *31* (7), 2068–2073.
- (100) Main, A. L.; Harvey, T. S.; Baron, M.; Boyd, J.; Campbell, I. D. The Three-Dimensional Structure of the Tenth Type III Module of Fibronectin: An Insight into RGD-Mediated Interactions. *Cell* **1992**, *71* (4), 671–678.
- (101) Carr, P. A.; Erickson, H. P.; Palmer, A. G. Backbone Dynamics of Homologous Fibronectin Type III Cell Adhesion Domains from Fibronectin and Tenascin. *Structure* **1997**, *5* (7), 949–959.
- (102) Dickinson, C. D.; Veerapandian, B.; Dai, X.-P.; Hamlin, R. C.; Xuong, N.; Ruoslahti, E.; Ely, K. R. Crystal Structure of the Tenth Type {III} Cell Adhesion Molecule of Human Fibronectin. *J. Mol.*

Biol **1994**, 236, 1079–1092.

(103) Obara, M.; Kang, M. S.; Yamada, K. M. Site-Directed Mutagenesis of the Cell-Binding Domain of Human Fibronectin: Separable, Synergistic Sites Mediate Adhesive Function. *Cell* **1988**, 53 (4), 649–657.

(104) Aota, S.; Nagai, T.; Olden, K.; Akiyama, S. K.; Yamada, K. M. Fibronectin and Integrins in Cell Adhesion and Migration. *Biochem Soc Trans* **1991**, 19 (4), 830–835.

(105) Nagai, T.; Yamakawa, N.; Aota, S.; Yamada, S. S.; Akiyama, S. K.; Olden, K.; Yamada, K. M. Monoclonal Antibody Characterization of Two Distant Sites Required for Function of the Central Cell-Binding Domain of Fibronectin in Cell Adhesion, Cell Migration, and Matrix Assembly. *J. Cell Biol.* **1991**, 114 (6), 1295–1305.

(106) Bowditch, R. D.; Hariharan, M.; Tominna, E. F.; Smith, J. W.; Yamada, K. M.; Getzoff, E. D.; Ginsberg, M. H. Identification of a Novel Integrin Binding-Site in Fibronectin - Differential Utilization By Beta-3 Integrins. *J. Biol. Chem.* **1994**, 269 (14), 10856–10863.

(107) Aota, S. I.; Nomizu, M.; Yamada, K. M. The Short Amino Acid Sequence Pro-His-Ser-Arg-Asn in Human Fibronectin Enhances Cell-Adhesive Function. *J. Biol. Chem.* **1994**, 269 (40), 24756–24761.

(108) Mould, A. P.; Askari, J. A.; Aota, S.; Yamada, K. M.; Irie, A.; Takada, Y.; Mardon, H. J.; Humphries, M. J. Defining the Topology of Integrin $\alpha 5 \beta 1$ -Fibronectin Interactions Using Inhibitory Anti- $\alpha 5$ and Anti- $\beta 1$ Monoclonal Antibodies. *J. Biol. Chem.* **1997**, 272 (28), 17283–17292.

(109) Mould, A. P.; Symonds, E. J. H.; Buckley, P. A.; Grossmann, J. G.; McEwan, P. A.; Barton, S. J.; Askari, J. A.; Craig, S. E.; Bella, J.; Humphries, M. J. Structure of an Integrin-Ligand Complex Deduced from Solution X-Ray Scattering and Site-Directed Mutagenesis. *J. Biol. Chem.* **2003**, 278 (41), 39993–39999.

(110) Adair, B. D.; Xiong, J. P.; Maddock, C.; Goodman, S. L.; Arnaout, M. A.; Yeager, M. Three-Dimensional EM Structure of the Ectodomain of Integrin $\alpha V \beta 3$ in a Complex with Fibronectin. *J.*

1 *Cell Biol.* **2005**, 168 (7), 1109–1118.

2 (111) Grant, R. P.; Spitzfaden, C.; Altroff, H.; Campbell, I. D.; Mardon, H. J. Structural Requirements for
3 Biological Activity of the Ninth and Tenth FIII Domains of Human Fibronectin. *J. Biol. Chem.* **1997**,
4 272 (10), 6159–6166.

5 (112) Copie, V.; Tomita, Y.; Akiyama, S. K.; Aota, S.; Yamada, K. M.; Venable, R. M.; Pastor, R. W.;
6 Krueger, S.; Torchia, D. A. Solution Structure and Dynamics of Linked Cell Attachment Modules
7 of Mouse Fibronectin Containing the RGD and Synergy Regions: Comparison with the Human
8 Fibronectin Crystal Structure. *J Mol Biol* **1998**, 277 (3), 663–682.

9 (113) Leahy, D. J.; Aukhil, I.; Erickson, H. P. 2.0 Å Crystal Structure of a Four-Domain Segment of Human
10 Fibronectin Encompassing the RGD Loop and Synergy Region. *Cell* **1996**, 84 (1), 155–164.

11 (114) Danen, E. H. J.; Aota, S.; van Kraats, A.; Yamada, K. M.; Dirk, J.; van Muijen, G. N. P.; van Kraats,
12 A. A. Requirement for the Synergy Site for Cell Adhesion to Fibronectin Depends on the Activation
13 State of Integrin $\alpha 5\beta 1$. **1995**, 270 (37), 1–8.

14 (115) Chen, J.; Maeda, T.; Sekiguchi, K.; Sheppard, D. Distinct Structural Requirements for Interaction of
15 the Integrins $\alpha 5\beta 1$, $\alpha v\beta 5$, and $\alpha v\beta 6$ with the Central Cell Binding Domain
16 in Fibronectin. *Cell Adhes. Commun.* **1996**, 4 (4–5), 237–250.

17 (116) Garcia, A. J.; J, T.; D, B. Two-Stage Activation for $\alpha 5\beta 1$ Integrin Binding to Surface-
18 Adsorbed Fibronectin. *J. Biol. Chem.* **1998**, 273 (52), 34710–34715.

19 (117) Redick, S. D.; Settles, D. L.; Briscoe, G.; Erickson, H. P. Defining Fibronectin ' S Cell Adhesion
20 Synergy Site by Site-Directed Mutagenesis. *J. Cell Biol.* **2000**, 149 (2), 521–527.

21 (118) Altroff, H.; Choulter, L.; Mardon, H. J. Synergistic Activity of the Ninth and Tenth FIII Domains of
22 Human Fibronectin Depends upon Structural Stability. *J. Biol. Chem.* **2003**, 278 (1), 491–497.

23 (119) Friedland, J. C.; Lee, M. H.; David, B. Mechanically Activated Integrin Switch Controls $\alpha 5\beta 1$

Function. *Science* (80-.). **2009**, 323 (5914), 642–644.

(120) Krammer, A.; Craig, D.; Thomas, W. E.; Schulten, K.; Vogel, V. A Structural Model for Force Regulated Integrin Binding to Fibronectin's RGD-Synergy Site. *Matrix Biol.* **2002**, 21 (2), 139–147.

(121) Singh, P.; Carraher, C.; Schwarzbauer, J. E. Assembly of Fibronectin Extracellular Matrix. *Annu Rev Cell Dev Biol* **2010**, 26, 397–419.

(122) Zhong, C.; Chrzanowska-Wodnicka, M.; Brown, J.; Shaub, A.; Belkin, A. M.; Burridge, K. Rho-Mediated Contractility Exposes a Cryptic Site in Fibronectin and Induces Fibronectin Matrix Assembly. *J. Cell Biol.* **1998**, 141 (2), 539–551.

(123) Burridge, K.; Chrzanowska-Wodnicka, M. Focal Adhesions, Contractility, and Signaling. *Annu. Rev. Cell Dev. Biol.* **1996**, 12 (1), 463–519.

(124) Ali, I. U.; Hynes, R. O. Effects of Cytochalasin B and Colchicine on Attachment of a Major Surface Protein of Fibroblasts. *Biochim. Biophys. Acta* **1977**, 471, 16–24.

(125) Wu, C.; Keivens, V. M.; O'Toole, T. E.; McDonald, J. A.; Ginsberg, M. H. Integrin Activation and Cytoskeletal Interaction Are Essential for the Assembly of a Fibronectin Matrix. *Cell* **1995**, 83(5), 715–724.

(126) Christopher, R. A.; Kowalczyk, A. P.; McKeown-Longo, P. J. Localization of Fibronectin Matrix Assembly Sites on Fibroblasts and Endothelial Cells. *J. Cell Sci.* **1997**, 110 (5), 569–581.

(127) McDonald, J. A.; Quade, B. J.; Broekelmann, T. J.; LaChance, R.; Forsman, K.; Hasegawa, E.; Akiyama, S. Fibronectin's Cell-Adhesive Domain and an Amino-Terminal Matrix Assembly Domain Participate in Its Assembly into Fibroblast Pericellular Matrix. *J. Biol. Chem.* **1987**, 262 (7), 2957–2967.

(128) Roman, J.; LaChance, R.; Broekelmann, T. J.; Kennedy, C. J. R.; Wayner, E. A.; Carter, W. G.; McDonald, J. A. The Fibronectin Receptor Is Organized by Extracellular Matrix Fibronectin:

Implications for Oncogenic Transformation and for Cell Recognition of Fibronectin Matrices. *J. Cell Biol.* **1989**, *108*, 2529–2453.

(129) Fogerty, F. J.; Akiyama, S. K.; Yamada, K. M.; Mosher, D. E. Inhibition of Binding of Fibronectin to Matrix Assembly Sites by Anti-Integrin (Alpha 5 Beta 1) Antibodies. *J. Cell Biol.* **1990**, *111* (2), 699–708.

(130) Wu, C.; Bauer, J. S.; Juliano, R. L.; McDonald, J. A. The Alpha 5 Beta 1 Integrin Fibronectin Receptor, but Not the Alpha 5 Cytoplasmic Domain, Functions in an Early and Essential Step in Fibronectin Matrix Assembly. *J Biol Chem* **1993**, *268* (29), 21883–21888.

(131) Huveneers, S.; Truong, H.; Fassler, R.; Sonnenberg, A.; Danen, E. H. J. Binding of Soluble Fibronectin to Integrin $\alpha 5 \beta 1$ - Link to Focal Adhesion Redistribution and Contractile Shape. *J. Cell Sci.* **2008**, *121* (15), 2452–2462.

(132) Pankov, R.; Cukierman, E.; Katz, B. Z.; Matsumoto, K.; Lin, D. C.; Lin, S.; Hahn, C.; Yamada, K. M. Integrin Dynamics and Matrix Assembly: Tensin-Dependent Translocation of $\alpha 5 \beta 1$ Integrins Promotes Early Fibronectin Fibrillogenesis. *J. Cell Biol.* **2000**, *148* (5), 1075–1090.

(133) Lemmon, C. A.; Chen, C. S.; Romer, L. H. Cell Traction Forces Direct Fibronectin Matrix Assembly. *Biophys. J.* **2009**, *96* (2), 729–738.

(134) Mao, Y.; Schwarzbauer, J. E. Fibronectin Fibrillogenesis, a Cell-Mediated Matrix Assembly Process. *Matrix Biol.* **2005**, *24* (6), 389–399.

(135) Schwarzbauer, J. E. Identification of the Fibronectin Sequences Required for Assembly of a Fibrillar Matrix. *J Cell Biol* **1991**, *113* (6), 1463–1473.

(136) Sechler, J. L.; Takada, Y.; Schwarzbauer, J. E. Altered Rate of Fibronectin Matrix Assembly by Deletion of the First Type III Repeats. *J Cell Biol* **1996**, *134* (2), 573–583.

(137) Chernousov, M. A.; Metsis, M. L.; Kotliansky, V. E. Studies of Extracellular Fibronectin Matrix

Formation with Fluoresceinated Fibronectin and Fibronectin Fragments. *FEBS Lett.* **1985**, 183 (2), 365–369.

(138) McKeown-Longo, P. J.; Mosher, D. F. Interaction of the 70,000-Mol-Wt Amino-Terminal Fragment of Fibronectin with the Matrix-Assembly Receptor of Fibroblasts. *J. Cell Biol.* **1985**, 100 (2), 364–374.

(139) Ichihara-Tanaka, K.; Maeda, T.; Titani, K.; Sekiguchi, K. Matrix Assembly of Recombinant Fibronectin Polypeptide Consisting of Amino-Terminal 70 kDa and Carboxyl-Terminal 37 kDa Regions. *FEBS Lett.* **1992**, 299 (2), 155–158.

(140) Ichihara-Tanaka, K.; Titani, K.; Sekiguchi, K. Role of the Carboxyl-Terminal Fib2 Domain in Fibronectin Matrix Assembly. *J. Cell Sci.* **1995**, 108, 907–915.

(141) McKeown-Longo, P. J.; Mosher, D. F. Binding of Plasma Fibronectin to Cell Layers of Human Skin Fibroblasts. *J. Cell Biol.* **1983**, 97, 466–472.

(142) Morla, A.; Ruoslahti, E. A Fibronectin Self-Assembly Site Involved in Fibronectin Matrix Assembly: Reconstruction in a Synthetic Peptide. *J. Cell Biol.* **1992**, 118 (2), 421–429.

(143) Chernousov, M. A.; Fogerty, F. J.; Kotliansky, V. E.; Mosher, D. F. Role of the I-9 and III-1 Modules of Fibronectin in Formation of an Extracellular Fibronectin Matrix. *J. Biol. Chem.* **1991**, 266 (17), 10851–10858.

(144) Sechler, J. L.; Rao, H.; Cumiskey, A. M.; Vega-Colón, I.; Smith, M. S.; Murata, T.; Schwarzbauer, J. E. A Novel Fibronectin Binding Site Required for Fibronectin Fibril Growth during Matrix Assembly. *J. Cell Biol.* **2001**, 154 (5), 1081–1088.

(145) Santas, A. J.; Peterson, J. A.; Halbleib, J. L.; Craig, S. E.; Humphries, M. J.; Pesciotta Peters, D. M. Alternative Splicing of the IIICS Domain in Fibronectin Governs the Role of the Heparin II Domain in Fibrillogenesis and Cell Spreading. *J. Biol. Chem.* **2002**, 277 (16), 13650–13658.

- 1 (146) Dzamba, B. J.; Peters, D. M. Arrangement of Cellular Fibronectin in Noncollagenous Fibrils in
2 Human Fibroblast Cultures. *J. Cell Sci.* **1991**, *100* (Pt 3), 605–612.
- 3 (147) Peters, D. M. P.; Chen, Y.; Zardi, L.; Brummel, S. Conformation of Fibronectin Fibrils Varies:
4 Discrete Globular Domains of Type III Repeats Detected. *Microsc Microanal* **1998**, *4* (4), 385–396.
- 5 (148) Gudzenko, T.; Franz, C. M. Studying Early Stages of Fibronectin Fibrillogenesis in Living Cells by
6 Atomic Force Microscopy. *Mol. Biol. Cell* **2015**, *26* (18), 3190–3204.
- 7 (149) Früh, S. M.; Schoen, I.; Ries, J.; Vogel, V. Molecular Architecture of Native Fibronectin Fibrils.
8 *Nat. Commun.* **2015**, *6*, 7275.
- 9 (150) Müller, C.; Stamov, D. R.; Werner, C.; Pompe, T. Nanoscale Characterization of Cell Receptors and
10 Binding Sites on Cell-Derived Extracellular Matrices. *Ultramicroscopy* **2012**, *118*, 44–52.
- 11 (151) Lemmon, C. A.; Weinberg, S. H. Multiple Cryptic Binding Sites Are Necessary for Robust
12 Fibronectin Assembly: An in Silico Study. *Sci. Rep.* **2017**, *7* (1), 1–12.
- 13 (152) Raitman, I.; Huang, M. L.; Williams, S. A.; Friedman, B.; Godula, K.; Schwarzbauer, J. E. Heparin-
14 Fibronectin Interactions in the Development of Extracellular Matrix Insolubility. *Matrix Biol.* **2017**.
- 15 (153) Stathakis, N. E.; Mosesson, M. W. Interactions among Heparin, Cold-Insoluble Globulin, and
16 Fibrinogen in Formation of the Heparin-Precipitable Fraction of Plasma. *J. Clin. Invest.* **1977**, *60*
17 (4), 855–865.
- 18 (154) Richter, H.; Wendt, C.; Hormann, H. Aggregation and Fibril Formation of Plasma Fibronectin by
19 Heparin. *Biol Chem Hoppe Seyler* **1985**, *366* (5), 509–514.
- 20 (155) Benthley, K. L.; Klebes, R. J.; Hurstq, R. E.; Horowitzll, M. Heparin Binding Is Necessary, but Not
21 Sufficient, for Fibronectin Aggregation: A Fluorescence Polarization Study. *J. Biol. Chem.* **1985**,
22 *260* (12), 7250–7256.
- 23 (156) Izzard, C. S.; Radinsky, R.; Culp, L. A. Substratum Contacts and Cytoskeletal Reorganization of

- BALB / c 3T3 Cells on a Cell-Binding Fragment and Heparin- Binding Fragments of Plasma Fibronectin. *Exp. Cell Res.* **1986**, *165*, 320–336.
- (157) Woods, A.; McCarthy, J. B.; Furcht, L. T.; Couchman, J. R. A Synthetic Peptide from the COOH-Terminal Heparin-Binding Domain of Fibronectin Promotes Focal Adhesion Formation. *Mol. Biol. Cell* **1993**, *4* (6), 605–613.
- (158) Kapila, Y. L.; Wang, S.; Johnson, P. W. Mutations in the Heparin Binding Domain of Fibronectin in Cooperation with the V Region Induce Decreases in pp125(FAK) Levels plus Proteoglycan-Mediated Apoptosis via Caspases. *J. Biol. Chem.* **1999**, *274* (43), 30906–30913.
- (159) Bloom, L.; Ingham, K. C.; Hynes, R. O. Fibronectin Regulates Assembly of Actin Filaments and Focal Contacts in Cultured Cells via the Heparin-Binding Site in Repeat III13. *Mol. Biol. Cell* **1999**, *10* (5), 1521–1536.
- (160) Woods, A.; Longley, R. L.; Tumova, S.; Couchman, J. R. Syndecan-4 Binding to the High Affinity Heparin-Binding Domain of Fibronectin Drives Focal Adhesion Formation in Fibroblasts. *Arch Biochem Biophys* **2000**, *374* (1), 66–72.
- (161) Lin, F.; Ren, X. D.; Doris, G.; Clark, R. A. F. Three-Dimensional Migration of Human Adult Dermal Fibroblasts from Collagen Lattices into Fibrin/fibronectin Gels Requires Syndecan-4 Proteoglycan. *J. Invest. Dermatol.* **2005**, *124* (5), 906–913.
- (162) Mohri, H.; Tanabe, J.; Katoh, K.; Okubo, T. Identification of a Novel Binding Site to the Integrin $\alpha 5 \beta 1$ Located in the C-Terminal Heparin-Binding Domain of Human Plasma Fibronectin. *J Biol Chem* **1996**, *271* (26), 15724–15728.
- (163) Haugen, P.; Letourneau, P.; Drake, S.; LT. A Cell-Surface Heparan Sulfate Proteoglycan Mediates Neural Cell Adhesion and Spreading on … *J. Neurosci.* **1992**, *12* (July), 2597–1608.
- (164) Huebsch, J. C.; McCarthy, J. B.; Diglio, C. A.; Mooradian, D. L. Endothelial Cell Interactions With Synthetic Peptides From the Carboxyl-Terminal Heparin-Binding Domains of Fibronectin. *Circ.*

1 *Res.* **1995**, 77, 43–53.

2 (165) Viji, R. I.; Kumar, V. B. S.; Kiran, M. S.; Sudhakaran, P. R. Angiogenic Response of Endothelial
3 Cells to Heparin-Binding Domain of Fibronectin. *Int. J. Biochem. Cell Biol.* **2008**, 40 (2), 215–226.

4 (166) Stepp, M. A.; Daley, W. P.; Bernstein, A. M.; Pal-Ghosh, S.; Tadvalkar, G.; Shashurin, A.; Palsen,
5 S.; Jurjus, R. A.; Larsen, M. Syndecan-1 Regulates Cell Migration and Fibronectin Fibril Assembly.
6 *Exp. Cell Res.* **2010**, 316 (14), 2322–2339.

7 (167) Saunders, S.; Bernfield, M. Cell Surface Proteoglycan Binds Mouse Mammary Epithelial Cells to
8 Fibronectin and Behaves as a Receptor for Interstitial Matrix. *J. Cell Biol.* **1988**, 106 (2), 423–430.

9 (168) Dalton, B. a; McFarland, C. D.; Underwood, P. a; Steele, J. G. Role of the Heparin Binding Domain
10 of Fibronectin in Attachment and Spreading of Human Bone-Derived Cells. *J. Cell Sci.* **1995**, 108 (
11 Pt 5, 2083–2092.

12 (169) McCarthy, J. B.; Chelberg, M. K.; Mickelson, D. J.; Furcht, L. T. Localization and Chemical
13 Synthesis of Fibronectin Peptides with Melanoma Adhesion and Heparin Binding Activities.
14 *Biochemistry* **1988**, 27, 1380–1388.

15 (170) McCarthy, J. B.; Skubitz, A. P. N.; Zhao, Q.; Yi, X.; Mickelson, D. J.; Klein, D. J.; Furcht, L. T.
16 RGD-Independent Cell Adhesion to the Carboxy-Terminal Heparin-Binding Fragment of
17 Fibronectin Involves Heparin-Dependent and -Independent Activities. *J. Cell Biol.* **1990**, 110 (3),
18 777–787.

19 (171) Drake, S. L.; Klein, D. J.; Mickelson, D. J.; Oegema, T. R.; Furcht, L. T.; McCarthy, J. B.; Drake,
20 S. L.; Klein, D. J.; Mickelson, D. J.; Oegema, T. R.; et al. Cell Surface Phosphatidylinositol-
21 Anchored Heparan Sulfate Proteoglycan Initiates Mouse Melanoma Cell Adhesion to a Fibronectin-
22 Derived, Heparin-Binding Synthetic Peptide. *J. Cell Biol.* **1992**, 117 (6), 1331–1341.

23 (172) Mostafavi-Pour, Z.; Askari, J. A.; Whittard, J. D.; Humphries, M. J. Identification of a Novel
24 Heparin-Binding Site in the Alternatively Spliced IIICS Region of Fibronectin: Roles of Integrins

- and Proteoglycans in Cell Adhesion to Fibronectin Splice Variants. *Matrix Biol.* **2001**, *20* (1), 63–73.
- (173) Haugen, P. K.; McCarthy, J. B.; Skubitz, A. P. N.; Furcht, L. T.; Letourneau, P. C. Recognition of the A Chain Carboxy-Terminal Heparin Binding Region of Fibronectin Involves Multiple Sites: Two Contiguous Sequences Act Independently to Promote Neural Cell Adhesion. *J. Cell Biol.* **1990**, *111* (6 I), 2733–2745.
- (174) Yoneda, J.; Saiki, I.; Igarashi, Y.; Kobayashi, Hideo; Fujii, H.; Ishizaki, Y.; Kimizuka, F.; Kato, I.; Azuma, I. Role of the Heparin-Binding Domain of Chimeric Peptides Derived from Fibronectin in Cell Spreading and Motility. *Exp. Cell Res.* **1995**, *217*, 169–179.
- (175) Kusano, Y.; Oguri, K.; Nagayasu, Y.; Munesue, S.; Ishihara, M.; Saiki, I.; Yonekura, H.; Yamamoto, H.; Okayama, M. Participation of Syndecan 2 in the Induction of Stress Fiber Formation in Cooperation with Integrin $\alpha 5 \beta 1$: Structural Characteristics of Heparan Sulfate Chains with Avidity to COOH-Terminal Heparin-Binding Domain of Fibronectin. *Exp. Cell Res.* **2000**, *256* (2), 434–444.
- (176) Zhang, Y.; Lu, H.; Dazin, P.; Kapila, Y. Functional Differences between Integrin $\alpha 4$ and Integrins $\alpha 5 / \alpha v$ in Modulating the Motility of Human Oral Squamous Carcinoma Cells in Response to the V Region and Heparin-Binding Domain of Fibronectin. *Exp. Cell Res.* **2004**, *295* (1), 48–58.
- (177) Kwon, M. J.; Kim, Y.; Choi, Y.; Kim, S. H.; Park, S.; Han, I.; Kang, D. H.; Oh, E. S. The Extracellular Domain of Syndecan-2 Regulates the Interaction of HCT116 Human Colon Carcinoma Cells with Fibronectin. *Biochem. Biophys. Res. Commun.* **2013**, *431* (3), 415–420.
- (178) Mohri, H.; Katoh, K.; Iwamatsu, A.; Okubo, T. The Novel Recognition Site in the C-Terminal Heparin-Binding Domain of Fibronectin by Integrin $\alpha 4 \beta 1$ Receptor on HL-60 Cells. *Exp. Cell Res.* **1996**, *222* (2), 326–332.
- (179) Liao, Y.-F.; Gotwals, P. J.; Koteliansky, V. E.; Sheppard, D.; Van De Water, L. The EIIIA Segment of Fibronectin Is a Ligand for Integrins $\alpha 9 \beta 1$ and $\alpha 4 \beta 1$ Providing a Novel Mechanism for

Regulating Cell Adhesion by Alternative Splicing. *J. Biol. Chem.* **2002**, 277 (17), 14467–14474.

(180) Ejim, O. S.; Blunn, G. W.; Brown, R. A. Production of Artificial-Orientated Mats and Strands from Plasma Fibronectin: A Morphological Study. *Biomaterials* **1993**, 14 (10), 743–748.

(181) Mitsi, M.; Handschin, S.; Gerber, I.; Schwartländer, R.; Klotzsch, E.; Wepf, R.; Vogel, V. The Ultrastructure of Fibronectin Fibers Pulled from a Protein Monolayer at the Air-Liquid Interface and the Mechanism of the Sheet-to-Fiber Transition. *Biomaterials* **2015**, 36, 66–79.

(182) Spatz, J. P.; Ulmer, J.; Geiger, B. Force-Induced Fibronectin Fibrillogenesis in Vitro. *Soft Matter* **2008**, 4 (10), 1998–2007.

(183) Feinberg, A. W.; Parker, K. K. Surface-Initiated Assembly of Protein Nanofabrics. *Nano Lett.* **2010**, 10 (6), 2184–2191.

(184) Mnatsakanyan, H.; Rico, P.; Grigoriou, E.; Candelas, A. M.; Rodrigo-Navarro, A.; Salmeron-Sanchez, M.; Sabater I Serra, R. Controlled Assembly of Fibronectin Nanofibrils Triggered by Random Copolymer Chemistry. *ACS Appl. Mater. Interfaces* **2015**, 7 (32), 18125–18135.

(185) Hocking, D. C.; Sottile, J.; McKeown-Longo, P. J. Fibronectin's III-1 Module Contains a Conformation-Dependent Binding Site for the Amino-Terminal Region of Fibronectin. *J. Biol. Chem.* **1994**, 269 (29), 19183–19191.

(186) Peters, D. M. P.; Chen, Y.; Zardi, L.; Brummel, S. Conformation of Fibronectin Fibrils Varies: Discrete Globular Domains of Type III Repeats Detected. *Microsc Microanal* **1998**, 4 (4), 385–396.

(187) Ingham, K. C.; Brew, S. A.; Huff, S.; Litvinovich, S. V. Cryptic Self-Association Sites in Type III Modules of Fibronectin. *J. Biol. Chem.* **1997**, 272 (3), 1718–1724.

(188) Morla, A.; Zhang, Z.; Ruoslahti, E. Superfibronectin Is a Functionally Distinct Form of Fibronectin. *Nature* **1994**, 367 (6459), 193–196.

(189) Pasqualini, R.; Bourfoulous, S.; Koivunen, E.; Woods, V. L.; Ruoslahti, E. A Polymeric Form of

- Fibronectin Has Antimetastatic Effects against Multiple Tumor Types. *Nat Med* **1996**, 2 (11), 1197–1203.
- (190) Yi, M.; Ruoslahti, E. A Fibronectin Fragment Inhibits Tumor Growth, Angiogenesis, and Metastasis. *Proc. Natl. Acad. Sci. U. S. A.* **2001**, 98 (2), 620–624.
- (191) Yi, M.; Sakai, T.; Fassler, R.; Ruoslahti, E. Antiangiogenic Proteins Require Plasma Fibronectin or Vitronectin for in Vivo Activity. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, 100 (20), 11435–11438.
- (192) Ohashi, T.; Erickson, H. P. Domain Unfolding Plays a Role in Superfibronectin Formation. *J. Biol. Chem.* **2005**, 280 (47), 39143–39151.
- (193) Stine, J. M.; Sun, Y.; Armstrong, G.; Bowler, B. E.; Briknarová, K. Structure and Unfolding of the Third Type III Domain from Human Fibronectin. *Biochemistry* **2015**, 54 (44), 6724–6733.
- (194) Stine, J. M.; Ahl, G. J. H.; Schlenker, C.; Rusnac, D. V.; Briknarová, K. The Interaction between the Third Type III Domain from Fibronectin and Anastellin Involves β -Strand Exchange. *Biochemistry* **2017**, 56 (35), 4667–4675.
- (195) Ohashi, T.; Erickson, H. P. Fibronectin Aggregation and Assembly: The Unfolding of the Second Fibronectin Type III Domain. *J. Biol. Chem.* **2011**, 286 (45), 39188–39199.
- (196) Briknarová, K.; Åkerman, M. E.; Hoyt, D. W.; Ruoslahti, E.; Ely, K. R. Anastellin, an FN3 Fragment with Fibronectin Polymerization Activity, Resembles Amyloid Fibril Precursors. *J. Mol. Biol.* **2003**, 332 (1), 205–215.
- (197) Schwarz-linek, U.; Werner, J. M.; Pickford, A. R.; Gurusiddappa, S.; Kim, J. H.; Pilka, E. S.; Briggs, J. A. G.; Gough, T. S.; Höök, M.; Campbell, I. D.; et al. Pathogenic Bacteria Attach to Human Fibronectin through a Tandem Beta-Zipper. *Nature* **2003**, 423, 5699–5702.
- (198) Litvinovich, S. V.; Brew, S. A.; Aota, S.; Akiyama, S. K.; Haudenschield, C.; Ingham, K. C. Formation of Amyloid-like Fibrils by Self-Association of a Partially Unfolded Fibronectin Type III Module. *J.*

Mol. Biol. **1998**, 280 (2), 245–258.

(199) Gee, E. P. S.; Yü Ksel, D.; Stultz, C. M.; Ingber, D. E. SLLISWD Sequence in the 10FNIII Domain Initiates Fibronectin Fibrillogenesis. *J. Biol. Chem.* **2013**, 288 (29), 21329–21340.

(200) Bascetin, R.; Admane, K.; Agniel, R.; Boudou, T.; Doussineau, T.; Antoine, R.; Gallet, O.; Leroy-Dudal, J.; Vendrely, C. Amyloid-like Aggregates Formation by Blood Plasma Fibronectin. *Int. J. Biol. Macromol.* **2017**, 97, 733–743.

(201) Bascetin, R.; Blay, L.; Kellouche, S.; Carreiras, F.; Picot, C.; Briand, M.; Agniel, R.; Gallet, O.; Vendrely, C.; Leroy-Dudal, J. Fibronectin Amyloid-like Aggregation Alters Its Extracellular Matrix Incorporation and Promotes a Single and Sparsed Cell Migration. *Exp. Cell Res.* **2018**.

(202) Bush, A. I. The Metallobiology of Alzheimer's Disease. *Trends Neurosci.* **2003**, 26 (4), 207–214.

(203) Frederickson, C. J.; Koh, J. Y.; Bush, A. I. The Neurobiology of Zinc in Health and Disease. *Nat. Rev. Neurosci.* **2005**, 6 (6), 449–462.

(204) Mantyh, P. W.; Ghilardi, J. R.; Rogers, S.; DeMaster, E.; Allen, C. J.; Stimson, E. R.; Maggio, J. E. Aluminum, Iron, and Zinc Ions Promote Aggregation of Physiological Concentrations of Beta-Amyloid Peptide. *J. Neurochem.* **1993**, 61, 1171–1174.

(205) Esler, W. P.; Stimson, E. R.; Jennings, J. M.; Ghilardi, J. R.; Mantyh, P. W.; Maggio, J. E. Zinc-Induced Aggregation of Human and Rat Beta-Amyloid Peptides in Vitro. *J Neurochem* **1996**, 66 (2), 723–732.

(206) Arispe, N.; Pollard, H. B.; Rojas, E. Zn²⁺ Interaction with Alzheimer Amyloid Beta Protein Calcium Channels. *Proc. Natl. Acad. Sci. U. S. A.* **1996**, 93, 1710–1715.

(207) Lovell, M. A.; Robertson, J. D.; Teesdale, W. J.; Campbell, J. L.; Markesbery, W. R. Copper, Iron and Zinc in Alzheimer's Disease Senile Plaques. *J. Neurol. Sci.* **1998**, 158 (1), 47–52.

(208) Danielsson, J.; Pierattelli, R.; Banci, L.; Gräslund, A. High-Resolution NMR Studies of the Zinc-

- 1 Binding Site of the Alzheimer's Amyloid β -Peptide. *FEBS J.* **2007**, 274 (1), 46–59.
- 2 (209) Lee, M. C.; Yu, W. C.; Shih, Y. H.; Chen, C. Y.; Guo, Z. H.; Huang, S. J.; Chan, J. C. C.; Chen, Y.
3 R. Zinc Ion Rapidly Induces Toxic, off-Pathway Amyloid- β Oligomers Distinct from Amyloid- β
4 Derived Diffusible Ligands in Alzheimer's Disease. *Sci. Rep.* **2018**, 8 (1), 1–16.
- 5 (210) McDonald, J. A. Extracellular Matrix Assembly. *Ann Rev Cell Biol* **1988**, 4, 183–207.
- 6 (211) Vuento, M.; Vartio, T.; Saraste, M.; von Bonsdorff, C. H.; Vaheri, A. Spontaneous and Polyamine-
7 Induced Formation of Filamentous Polymers from Soluble Fibronectin. *Eur J Biochem* **1980**, 105
8 (1), 33–42.
- 9 (212) Llopis-Hernandez, V.; Rico, P.; Moratal, D.; Altankov, G.; Salmeron-Sanchez, M. Role of Material-
10 Driven Fibronectin Fibrillogenesis in Cell Differentiation. *Biomaterials* **2011**, 32 (8), 2099–2105.
- 11 (213) Llopis-Hernández, V.; Cantini, M.; González-García, C.; Cheng, Z. A.; Yang, J.; Tsimbouri, P. M.;
12 García, A. J.; Dalby, M. J.; Salmerón-Sánchez, M. Material-Driven Fibronectin Assembly for High-
13 Efficiency Presentation of Growth Factors. *Sci. Adv.* **2016**, 2 (8), 1–10.
- 14 (214) Moulisová, V.; Gonzalez-García, C.; Cantini, M.; Rodrigo-Navarro, A.; Weaver, J.; Costell, M.;
15 Sabater i Serra, R.; Dalby, M. J.; García, A. J.; Salmerón-Sánchez, M. Engineered
16 Microenvironments for Synergistic VEGF – Integrin Signalling during Vascularization.
17 *Biomaterials* **2017**, 126, 61–74.
- 18 (215) Sun, Y.; Jallerat, Q.; Szymanski, J. M.; Feinberg, A. W. Conformal Nanopatterning of Extracellular
19 Matrix Proteins onto Topographically Complex Surfaces. *Nat Methods* **2015**, 12 (2), 134–136.
- 20 (216) Duffy, R. M.; Sun, Y.; Feinberg, A. W. Understanding the Role of ECM Protein Composition and
21 Geometric Micropatterning for Engineering Human Skeletal Muscle. *Ann. Biomed. Eng.* **2016**, 44
22 (6), 2076–2089.
- 23 (217) Palchesko, R. N.; Szymanski, J. M.; Sahu, A.; Feinberg, A. W. Shrink Wrapping Cells in a Defined

- Extracellular Matrix to Modulate the Chemo-Mechanical Microenvironment. *Cell Mol Bioeng* **2014**, 7 (3), 355–368.
- (218) Brown, R. A.; Blunn, G. W.; Ejim, O. S. Preparation of Orientated Fibrous Mats from Fibronectin: Composition and Stability. *Biomaterials* **1994**, 15 (6), 457–464.
- (219) Ahmed, Z.; Underwood, S.; Brown, R. A. A. Nerve Guide Material Made from Fibronectin: Assessment of in Vitro Properties. *Tissue Eng.* **2003**, 9 (2), 219–231.
- (220) Whitworth, I. H.; Brown, R. A.; Dore, C.; Green, C. J.; Terenghi, G. Orientated Mats Of Fibronectin Material For Use In Peripheral As A Conduit. *J Hand Surg Br* **1995**, 20 (B), 429–436.
- (221) King, V. R.; Henseler, M.; Brown, R. A.; Priestley, J. V. Mats Made from Fibronectin Support Oriented Growth of Axons in the Damaged Spinal Cord of the Adult Rat. *Exp. Neurol.* **2003**, 182 (2), 383–398.
- (222) Phillips, J. B.; King, V. R.; Ward, Z.; Porter, R. A.; Priestley, J. V.; Brown, R. A. Fluid Shear in Viscous Fibronectin Gels Allows Aggregation of Fibrous Materials for CNS Tissue Engineering. *Biomaterials* **2004**, 25 (14), 2769–2779.
- (223) King, V. R.; Phillips, J. B.; Hunt-Grubbe, H.; Brown, R.; Priestley, J. V. Characterization of Non-Neuronal Elements within Fibronectin Mats Implanted into the Damaged Adult Rat Spinal Cord. *Biomaterials* **2006**, 27 (3), 485–496.
- (224) King, V. R.; Hewazy, D.; Alovskaya, A.; Phillips, J. B.; Brown, R. A.; Priestley, J. V. The Neuroprotective Effects of Fibronectin Mats and Fibronectin Peptides Following Spinal Cord Injury in the Rat. *Neuroscience* **2010**, 168 (2), 523–530.
- (225) Underwood, S.; Afoke, A.; Brown, R. A.; MacLeod, A. J.; Ayazi Shamlou, P.; Dunnill, P. Wet Extrusion of Fibronectin-Fibrinogen Cables for Application in Tissue Engineering. *Biotechnol. Bioeng.* **2001**, 73 (4), 295–305.

- (226) Zhou, N. F.; Pethica, B. A. Monolayers of Human-Plasma Fibronectin at the Air-Water-Interface. *Langmuir* **1986**, 2 (1), 47–50.
- (227) Vogel, V. Fibronectin in a Surface-Adsorbed State - Insolubilization and Self-Assembly. *Proteins Interfaces II* **1995**, 602, 505–518.
- (228) Klotzsch, E.; Smith, M. L.; Kubow, K. E.; Muntwyler, S.; Little, W. C.; Beyeler, F.; Gourdon, D.; Nelson, B. J.; Vogel, V. Fibronectin Forms the Most Extensible Biological Fibers Displaying Switchable Force-Exposed Cryptic Binding Sites. *Proc. Natl. Acad. Sci. U. S. A.* **2009**, 106 (43), 18267–18272.
- (229) Rossier, O.; Cuvelier, D.; Borghi, N.; Puech, P. H.; Derenyi, I.; Buguin, A.; Nassoy, P.; Brochard-Wyart, F. Giant Vesicles under Flows: Extrusion and Retraction of Tubes. *Langmuir* **2003**, 19 (3), 575–584.
- (230) Evans, E.; Yeung, A. Hidden Dynamics in Rapid Changes of Bilayer Shape. *Chem. Phys. Lipids* **1994**, 73 (1–2), 39–56.
- (231) Campioni, S.; Carret, G.; Jordens, S.; Nicoud, L.; Mezzenga, R.; Riek, R. The Presence of an Air – Water Interface Affects Formation and Elongation of α - Synuclein Fibrils. *J. Am. Chem. Soc.* **2014**, 136, 2866–2875.
- (232) Little, W. C.; Smith, M. L.; Ebnetter, U.; Vogel, V. Assay to Mechanically Tune and Optically Probe Fibrillar Fibronectin Conformations from Fully Relaxed to Breakage. *Matrix Biol* **2008**, 27 (5), 451–461.
- (233) Mitsi, M.; Schulz, M. M. P.; Gousopoulos, E.; Ochsenbein, A. M.; Detmar, M.; Vogel, V. Walking the Line: A Fibronectin Fiber-Guided Assay to Probe Early Steps of (Lymph) Angiogenesis. *PLoS One* **2015**, 10 (12).

1
2
3
4
5
6
7
8
9
10
11

12
13
14
15
16

Table of Content Graphics