coatings

Supplementary Materials: Biochip Surfaces Containing Recombinant Cell-Binding Domains of Fibronectin

Martynas Gavutis [1,*], Gintarė Stankevičienė [1], Airina Mazėtytė-Godienė [1], Tadas Jelinskas [1], Jurgita Vinskienė [2], Perttu Haimi [2], Danas Baniulis [2] and Ramūnas Valiokas [1]

[1] Center for Physical Sciences and Technology, Department of Nanoengineering, Savanorių 231, LT-02300 Vilnius, Lithuania; gintare.stankeviciene@ftmc.lt (G.S.); airina.mazetyte@ftmc.lt (A.M.-G.); tadas.jelinskas@ftmc.lt (T.J.); valiokas@ftmc.lt (R.V.)
[2] Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture, Kaunas Str. 30, Babtai, LT-54333 Kaunas reg., Lithuania; jurgita.vinskiene@lammc.lt (J.V.); perttu-juhani.haimi@lammc.lt (P.H.); danas.baniulis@lammc.lt (D.B.)
* Correspondence: gavutis@ftmc.lt

Citation: Gavutis, M.; Stankevičienė, G.; Mazėtytė-Godienė, A.; Jelinskas, T.; Vinskienė, J.; Haimi, P.; Baniulis, D.; Valiokas, R. Biochip Surfaces Containing Recombinant Cell-Binding Domains of Fibronectin. *Coatings* **2022**, *12*, x. https://doi.org/10.3390/xxxxx

Academic Editor: Evgeniia S. Vikulova

Received: 17 May 2022
Accepted: 18 June 2022
Published: 21 June 2022

A. FnIII9-10: total 606 nt / CDS 576 nt / protein 192 a.a.

```
ggt ggt cat atg ctt gat tcc cca act ggc att gac ttt tct gat att act gcc aac tct
            M   L   D   S   P   T   G   I   D   F   S   D   I   T   A   N   S
ttt act gtg cac tgg att gct cct cga gcc acc atc act ggc tac agg atc cgc cat cat
 F   T   V   H   W   I   A   P   R   A   T   I   T   G   Y   R   I   R   H   H
ccc gag cac ttc agt ggg aga cct cga gaa gat cgg gtg ccc cac tct cgg aat tcc atc
 P   E   H   F   S   G   R   P   R   E   D   R   V   P   H   S   R   N   S   I
acc ctc acc aac ctc act cca ggc aca gag tat gtg gtc agc atc gtt gct ctt aat ggc
 T   L   T   N   L   T   P   G   T   E   Y   V   V   S   I   V   A   L   N   G
aga gag gaa agt ccc tta ttg att ggc caa caa tca aca gtt tct gat gtt ccg agg gac
 R   E   E   S   P   L   L   I   G   Q   Q   S   T   V   S   D   V   P   R   D
ctg gaa gtt gtt gct gcg acc ccc acc agc cta ctg atc agc tgg gat gct cct gct gtc
 L   E   V   V   A   A   T   P   T   S   L   L   I   S   W   D   A   P   A   V
aca gtg aga tat tac agg atc act tac gga gaa aca gga gga aat agc cct gtc cag gag
 T   V   R   Y   Y   R   I   T   Y   G   E   T   G   G   N   S   P   V   Q   E
ttc act gtg cct ggg agc aag tct aca gct acc atc agc ggc ctt aaa cct gga gtt gat
 F   T   V   P   G   S   K   S   T   A   T   I   S   G   L   K   P   G   V   D
tat acc atc act gtg tat gct gtc act ggc cgt gga gac agc ccc gca agc agc aag cca
 Y   T   I   T   V   Y   A   V   T   G   R   G   D   S   P   A   S   S   K   P
att tcc att aat tac cga aca gaa att gac aaa cca tcc cag atg tgc gga aga gca acc
 I   S   I   N   Y   R   T   E   I   D   K   P   S   Q   M
acc
```

B. FnIII10: total 324 nt / CDS 309 nt / protein 103 a.a.

```
cat atg gtt agt gat gtt ccg cgt gat ctg gaa gtt gtt gca gca acc ccg acc agc ctg
    M   V   S   D   V   P   R   D   L   E   V   V   A   A   T   P   T   S   L
ctg att agc tgg gat gca ccg gca gtt acc gtt cgt tat tat cgc att acc tat ggt gaa
 L   I   S   W   D   A   P   A   V   T   V   R   Y   Y   R   I   T   Y   G   E
acc ggt ggt aat agt ccg gtt caa gaa ttt acc gtt ccg ggt agc aaa agc acc gcc acc
 T   G   G   N   S   P   V   Q   E   F   T   V   P   G   S   K   S   T   A   T
att agc ggt ctg aaa ccg ggt gtt gat tac acc att aca gtt tat gcc gtt acc ggt cgt
 I   S   G   L   K   P   G   V   D   Y   T   I   T   V   Y   A   V   T   G   R
ggt gat tca ccg gca agc agc aaa ccg att agc att aac tat cgt acc gaa atc gat aaa
 G   D   S   P   A   S   S   K   P   I   S   I   N   Y   R   T   E   I   D   K
ccg tcc cag atg tgc gga aga gca
 P   S   Q   M
```

Figure S1. DNA sequences used for cloning of human fibronectin 1 (Fn1) type III domain fragments corresponding to domains 9-10 (**A**) and 10 (**B**). Restriction enzyme NdeI and LguI recognition sequences are marked in yellow and green, respectively.

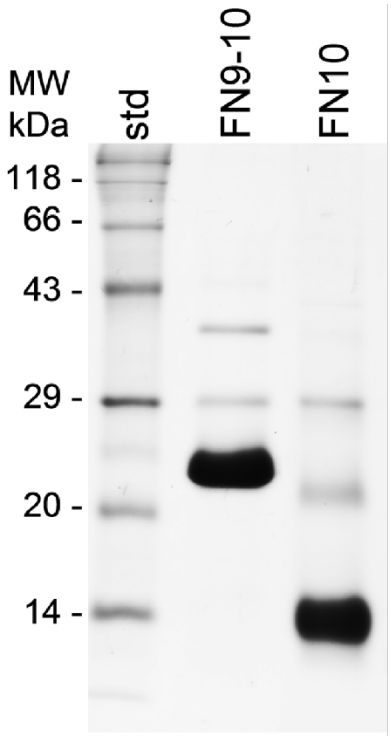
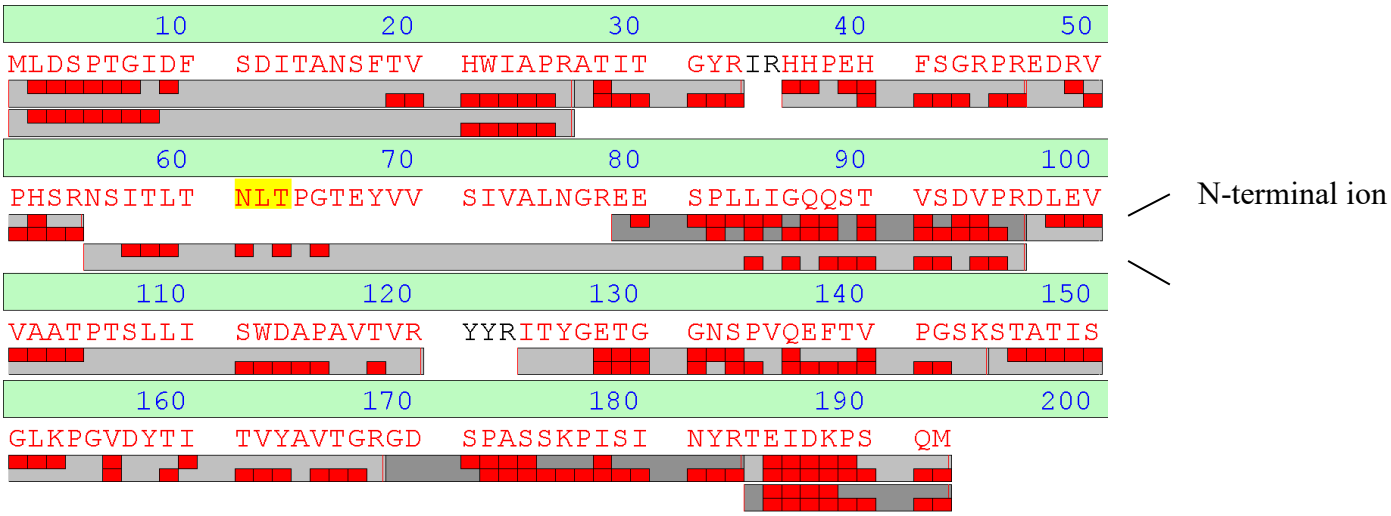


Figure S2. SDS PAGE separation of purified recombinant fibronectin fragments. Molecular weight (MW) marker (lane 1), and 5 μg of type-III repeat 9-10 (FnIII9-10) (lane 2) and 10 (FnIII10) (lane 3) fragments were separated on 12% polyacrylamide gel.

A.



B.

No.	m/z meas.	Δ m/z [ppm]	RMS90 [ppm]	Scores	Range	Sequence	Modifications
1	1266.6948	6.16	4.5	18.4 (M:18.4)	55 - 78	R.NSITLTNLTPGTEYVVSIVALNGR.E	
2	391.216	5.78	9.22	53.6 (M:53.6)	27 - 33	R.ATITGYR.I	
3	452.8972	7.5	7.86	24.8 (M:24.8)	36 - 46	R.HHPEHFSGRPR.E	
4	498.2576	6.15	13.6	24.9 (M:24.9)	47 - 54	R.EDRVPHSR.N	
5	524.7565	7.48	6.01	46.2 (M:46.2)	184 - 192	R.TEIDKPSQM.-	
6	532.7534	6.26	8.54	68.8 (M:68.8)	184 - 192	R.TEIDKPSQM.-	Oxidation: 9
7	796.413	7.12	4.66	90.9 (M:90.9)	169 - 183	R.GDSPASSKPISINYR.T	
8	824.1157	5.51	5.24	67.2 (M:67.2)	145 - 168	K.STATISGLKPGVDYTITVYAVTGR.G	
9	842.1307	4.71	5.07	47.4 (M:47.4)	97 - 120	R.DLEVVAATPTSLLISWDAPAVTVR.Y	
10	964.4784	6.01	4.26	69.4 (M:69.4)		-.MLDSPTGIDFSDITANSFTVHWIAPR.A	
11	969.8102	6.1	4.59	58.5 (M:58.5)		-.MLDSPTGIDFSDITANSFTVHWIAPR.A	Oxidation: 1
12	978.0131	5.56	3.87	83.4 (M:83.4)	79 - 96	R.EESPLLIGQQSTVSDVPR.D	
13	1084.5388	9.11	4.97	71.2 (M:71.2)	124 - 144	R.ITYGETGGNSPVQEFTVPGSK.S	
14	1117.8441	-0.56	5.46	24.9 (M:24.9)	55 - 96	R.NSITLTNLTPGTEYVVSIVALNGREESPLLIG QQSTVSDVPR.D	

Figure S3. Characterization of recombinant fibronectin fragment Fn9-10 using mass spectrometry. The purified fragment was separated by SDS PAGE, the corresponding molecular weight band was excised and in-gel digested with trypsin. Tryptic peptide mass fingerprinting was carried out using the MASCOT server (Matrix science, London, UK) and corresponding peptide sequences. Data processed and visualized using the ProteinScape software (Bruker, Billerica, MA, USA). (**A**): Sequence map. The residues matching and not matching database sequence are shown in red and grey font colour, respectively. Gray bars indicate the identified peptides and the relative TIC peak intensity is indicated by the darkness of the shading (the darker the shading, the higher the intensity value). Red boxes indicate observed fragment ions that correspond to the sequence. The potential site of N-glycosylation is marked in yellow. (**B**): Peptide results table. The m/z-measured mass-to-charge ratio, Δm/z-difference in mass-to-charge ratio, RMS90-root mean square 90% confidence value, Scores–Mascot protein score, Range-position in protein (aa number to aa number), Sequence-peptide sequence, Modifications-chemical modifications present.