Supporting Information

Fiber Morphology

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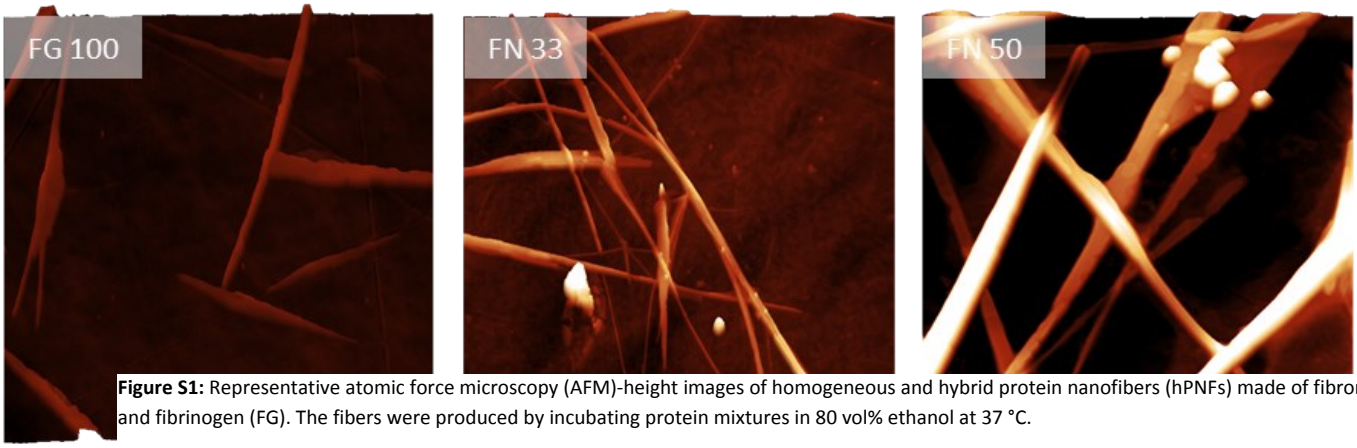
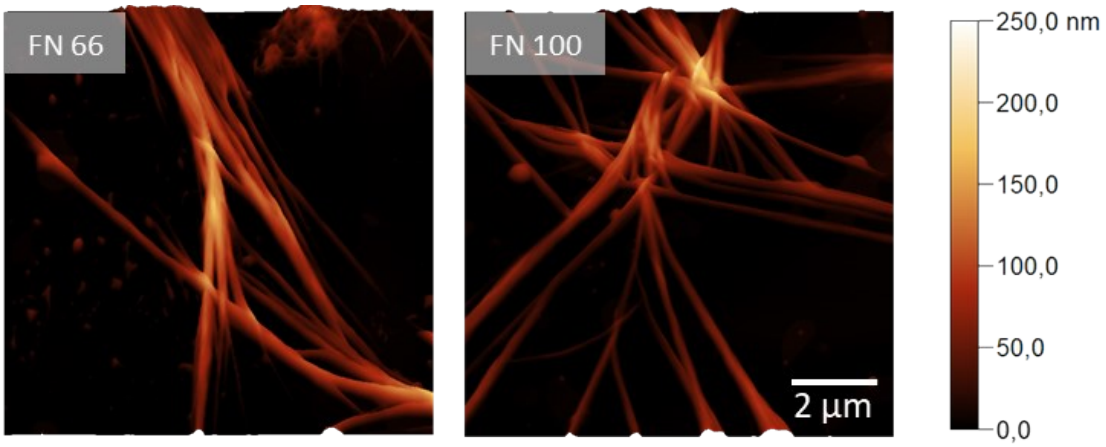


Figure S1: Representative atomic force microscopy (AFM)-height images of homogeneous and hybrid protein nanofibers (hPNFs) made of fibronectin (FN) and fibrinogen (FG). The fibers were produced by incubating protein mixtures in 80 vol% ethanol at 37 °C.



Supporting Information for: Self-assembled Fibrinogen-Fibronectin Hybrid Protein Nanofibers with Medium-Sensitive Stability

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Preliminary parameter studies on composition-dependent fiber formation

Prior to the investigation of the fibers' morphology and properties, we performed a parameter study to determine self-assembly conditions that reproducibly yield a high amount of fibers with little to no other structures, such as protein film or agglomerates. The results are displayed in Table T_S1. The assembly times were set to 1 h, 4 h and 7 d respectively. To test for fiber formation, AFM investigations of each sample were performed. A total amount of six batches has been investigated before we chose the assembly conditions for our main work.

Table T_S1: Overview of preliminary self-assembly tests for various FN:FG ratios. The color code shows the result of the assembly tests. The black arrow indicates the condition that we choose to continue with.

			FN:FG (mol/mol)						
T (°C)	C (ng µL^{-1})	t	1:0	4:1	2:1	1:1	1:2	1:4	0:1
37	20	1 h							
		4 h							
		7d							

	Pronounced and reproducible fiber formation, little to no aggregates
	Formation of fibers and aggregates, low reproducibility
	Formation of protein film and network like structures
	Formation of protein film and aggregates, no fibers observed

hPNF stability in PBS

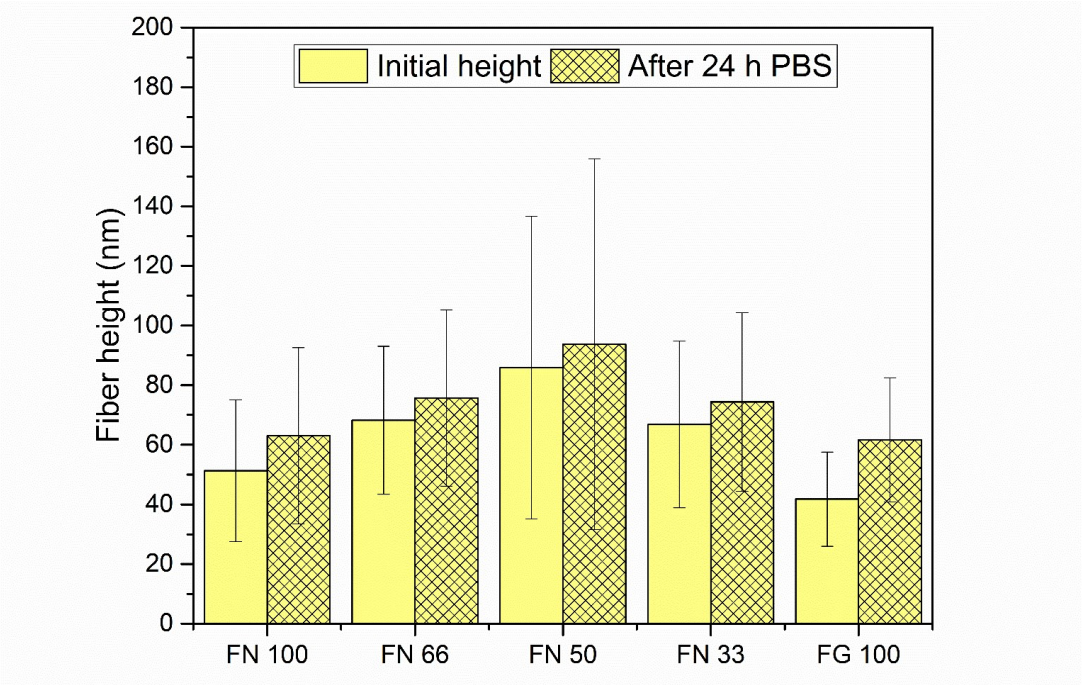

Figure S2: Average fiber heights of hPNFs, measured in the dry state *via* AFM before and after immersing the fiber coated substrates for 24 h in PBS. The error bars represent the standard deviation of the measured fiber heights.

Comparison of FN and FG amino acid sequences

The figures **Fig. S3 – S5** are showing relevant amino acids for protein interaction and amino acid sequences of human plasma fibronectin (FINC_HUMAN) and the alpha, beta and gamma chains of human plasma fibrinogen (FIBA_HUMAN, FIBB_HUMAN, FIBG_HUMAN) according to the uniprot alignment tool.[a] The amino acids in Figure **S6** were drawn with the PepDraw tool.[b] Identical regions are marked with red boxes, whereas the symbols in the third row signalize the degree of similarity: similar regions are marked with ".", very similar regions are marked with ":" and identical regions are marked with "*". Additionally, hydrophobic sequences are highlighted purple, while negatively charged sequences are highlighted in green and positively charged in yellow. The figures point out that the primary structures of FG and FN are matching, especially in the first third and (in case of FIBA_HUMAN) in the last third of the proteins primary structure. Correspondence of hydrophobic amino acid sequences and of amino acids with opposing charge can also be observed in these regions. According to Uniprot[a], FN contains a total amount of 496 charged amino acids of which 240 are negatively and 256 are positively charged while FG contains 460 charged amino acids with 213 being negative and 247 being positive. All of this aligns well with the observations we made earlier for the system of human plasma hemoglobin and albumin.[1] We hence assume that, by heat and ethanol induced partial protein unfolding, these amino acid sequences where able to interact with each other during fiber self-assembly.

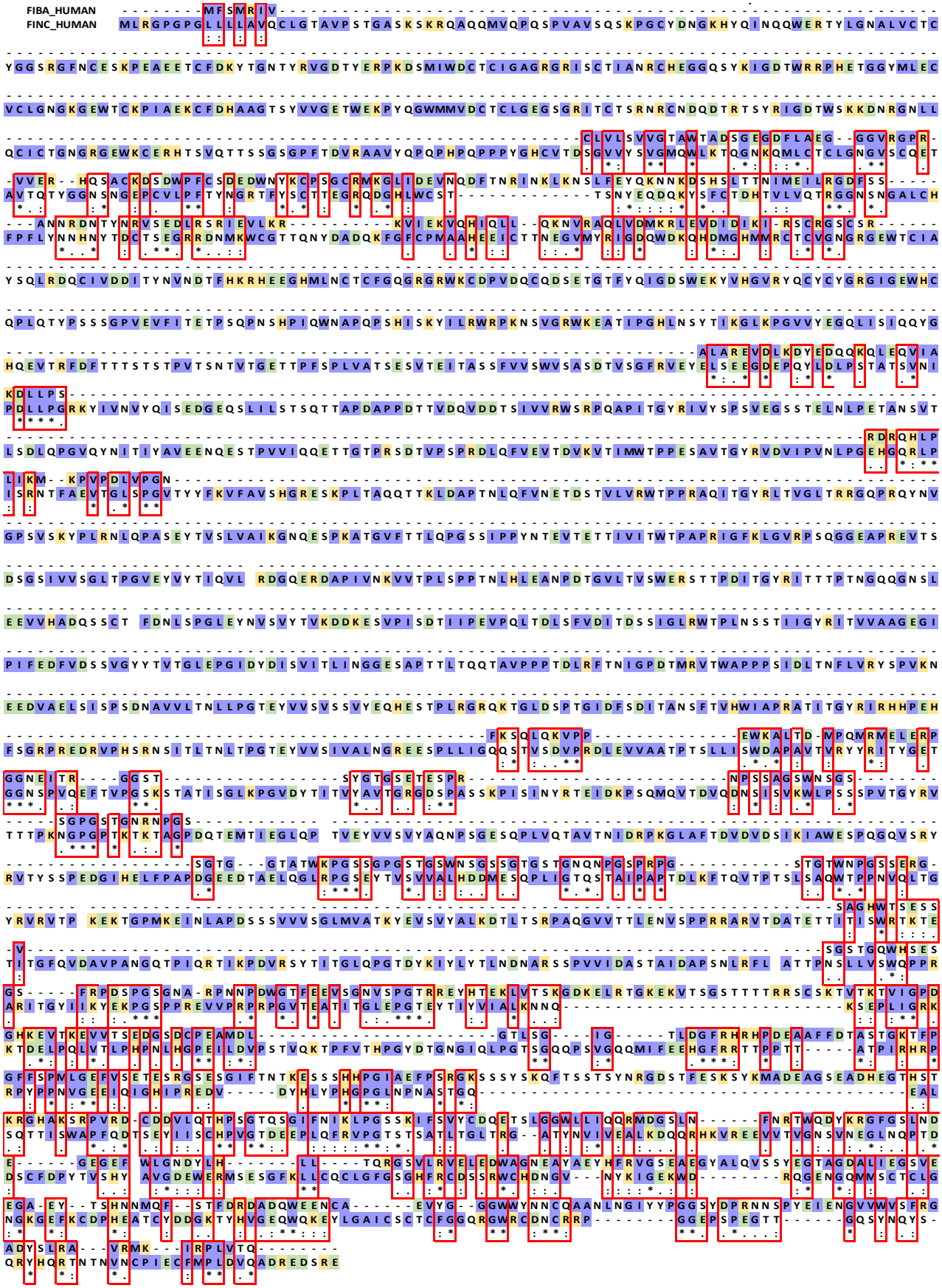

Figure S3: Sequence alignment on FIBA_HUMAN and FINC_HUMAN. Red boxes mark similar amino acids. Hydrophobic amino acids are highlighted with purple, negatively charged are highlighted with green and positively charged are highlighted with yellow.

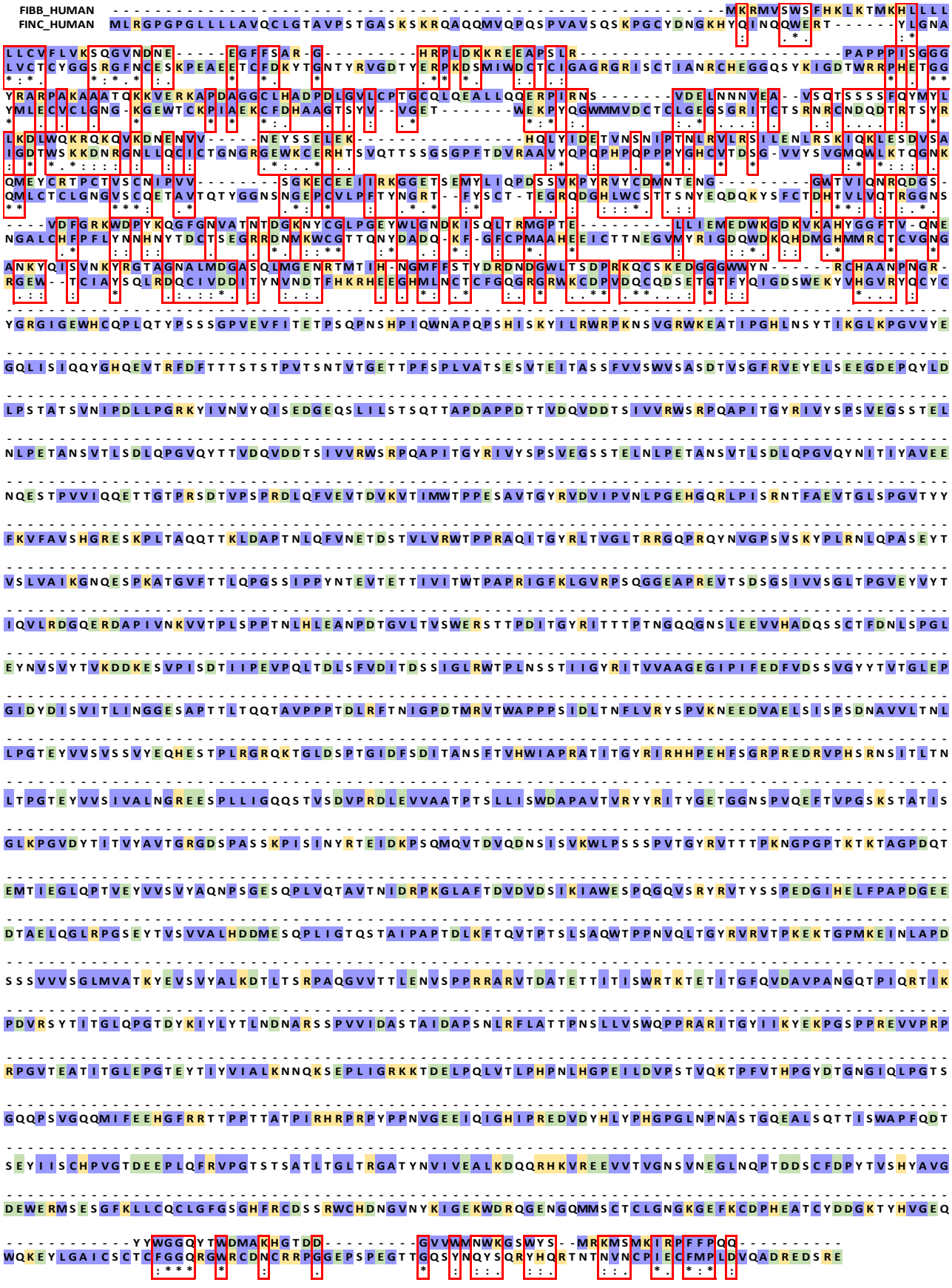

Figure S4: Sequence alignment on FIBB_HUMAN and FINC_HUMAN. Red boxes mark similar amino acids. Hydrophobic amino acids are highlighted with purple, negatively charged are highlighted with green and positively charged are highlighted with yellow.

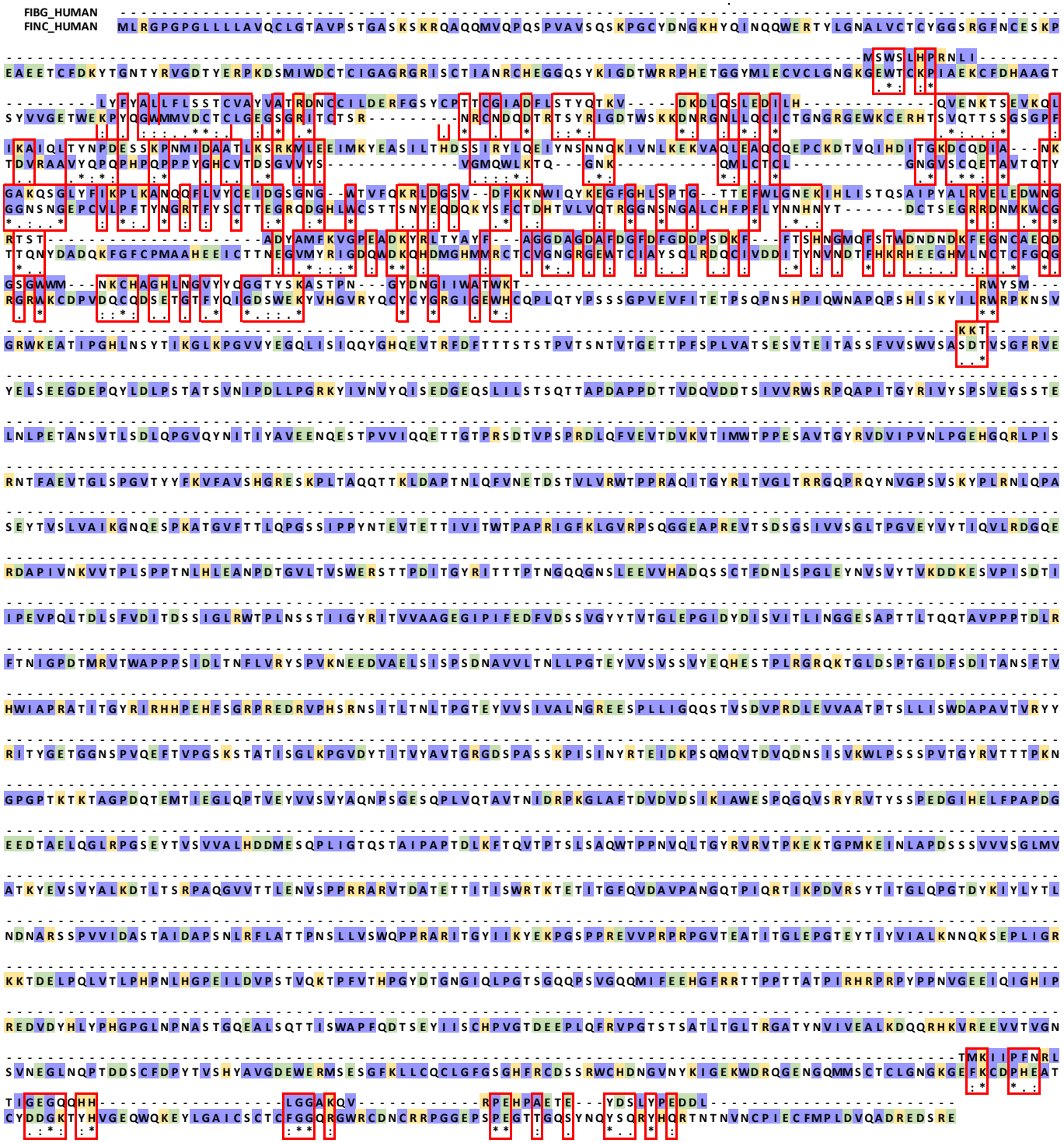

Figure S5: Sequence alignment on FIBG_HUMAN and FINC_HUMAN. Red boxes mark similar amino acids. Hydrophobic amino acids are highlighted with purple, negatively charged are highlighted with green and positively charged are highlighted with yellow.

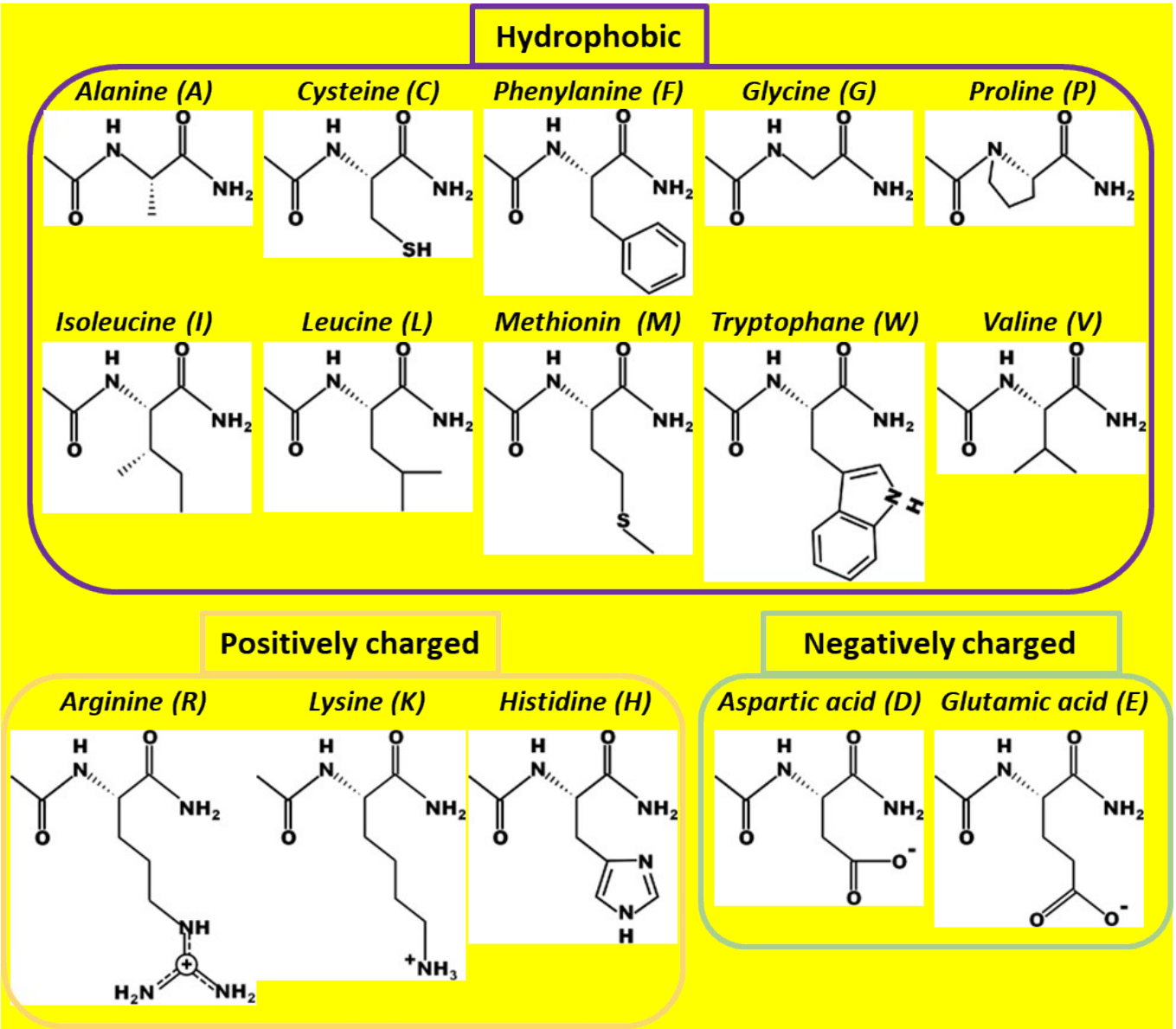

Figure **S6**: Structure formulas of hydrophobic (purple), positively (yellow) and negatively (green) charged amino acid side groups (according to Figures **S3 – S5**)

References:

1. C. Helbing, T. Deckert-Gaudig, I. Firkowska-Boden, G. Wei, V. Deckert and K. D. Jandt, *ACS Nano*, 2018, **12**, 1211-1219.

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[a] https://www.uniprot.org/align/

[b] http://pepdraw.com/