186. Composition & methods for the inhibition of fibrosis

(Wisconsin Alumni Research Foundation)

5TH KDDF GLOBAL C&D TECH FAIR

Asset Overview

Product Type	Peptide
Disease Area	Others
Indication	Fibrosis
Current Stage	Lead Optimization
Target	Fibronectin fiber deposition
MoA	A PEGylated functional upstream domain peptide (PEG-FUD), which tightly binds to fibronectin and inhibits the development of the fine fibrils that precede collagen deposits, preventing the formation of scar tissue.
Brief Description	• The FUD peptide was recombinantly expressed, purified, and PEGylated at the N-terminus using 10 kDa, 20 kDa, and 40 kDa methoxy-PEG aldehyde. The PEGylates were purified and fractionated using ionexchange chromatography. The molecular weight and degree of PEGylation of each conjugate was verified using MALDITOF. • The binding affinity of each PEG-FUD conjugate was studied using isothermal titration colorimetry (ITC) and their inhibitory potency was characterized by a cell-based matrix assembly in vitro assay. • The 10 kDa, 20 kDa, and 40 kDa PEG-FUD conjugates were synthesized and isolated in good purity as determined by HPLC analysis. Their molecular weight was consistent with attachment of a single PEG molecule to one FUD peptide. • The binding affinity (Kd) and the fibronectin fibrillogenesis inhibitory potency (IC50) of all PEG-FUD conjugates remained nanomolar and unaffected by the addition of PEG.
Intellectual Property	US10828372B2
Publication	Characterization of the PEGylated Functional Upstream Domain Peptide (PEG-FUD): a Potent Fibronectin Assembly Inhibitor with Potential as an Anti-Fibrotic Therapeutic. Pharmaceutical Research, (2018)
Inventors	Bianca R. Tomasini-Johansson, Glen S. Kwon, Pawel Waldemar Zbyszynski, Nathan Sandbo, Ksenija Bernau

Highlights

- The FUD peptide was successfully conjugated with 10 kDa, 20 kDa, or 40 kDa PEG moieties and isolated in good purity. The mass of the PEG-FUD constructs agreed with attachment of a single PEG molecule.
- Retention of low nanomolar binding affinity was found following PEGylation with all three constructs.
- All three PEG-FUD peptides were found to be equally effective at inhibiting FN fibrillogenesis in vitro compared to unmodified FUD.
- Anti-fibrotic value of this peptide and stress the importance of evaluation of these PEG-FUD constructs in the context of therapeutic efficacy and pharmacokinetic performance in an animal model of fibrosis.

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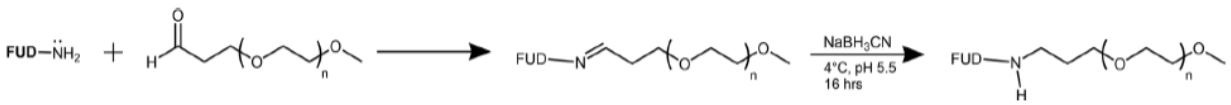
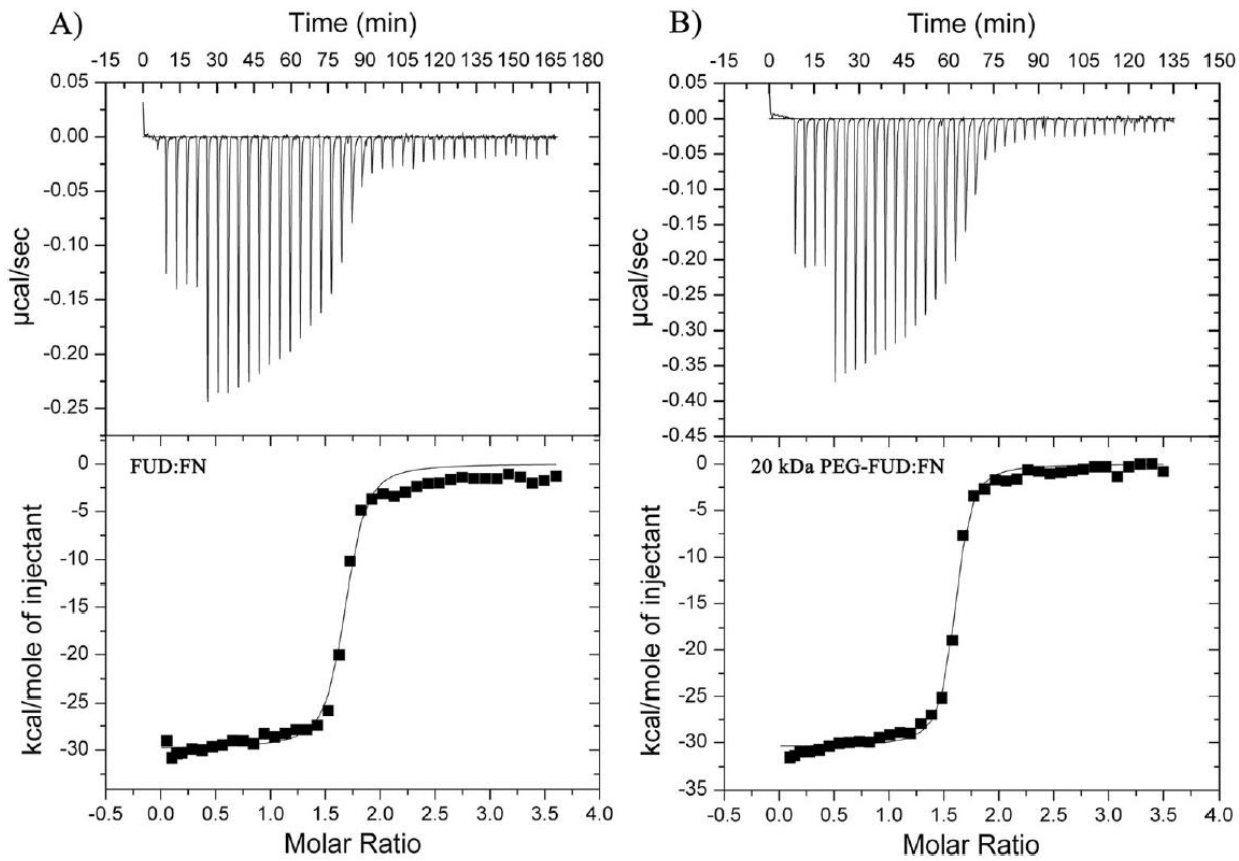
Key Data

The 10 kDa, 20 kDa, and 40 kDa PEGylated FUD constructs



Determination of binding affinity (Kd) and other thermodynamic parameters



Determination of binding affinity (Kd) and other thermodynamic parameters using Isothermal Titration Calorimetry (ITC). All experiments were performed using pH 7.4 PBS, 25°C chamber conditions, and human plasma FN. A) FUD into FN and B) 20 kDa PEG-FUD into FN experiment sample isotherm and thermograph. Each experiment was repeated in triplicates.

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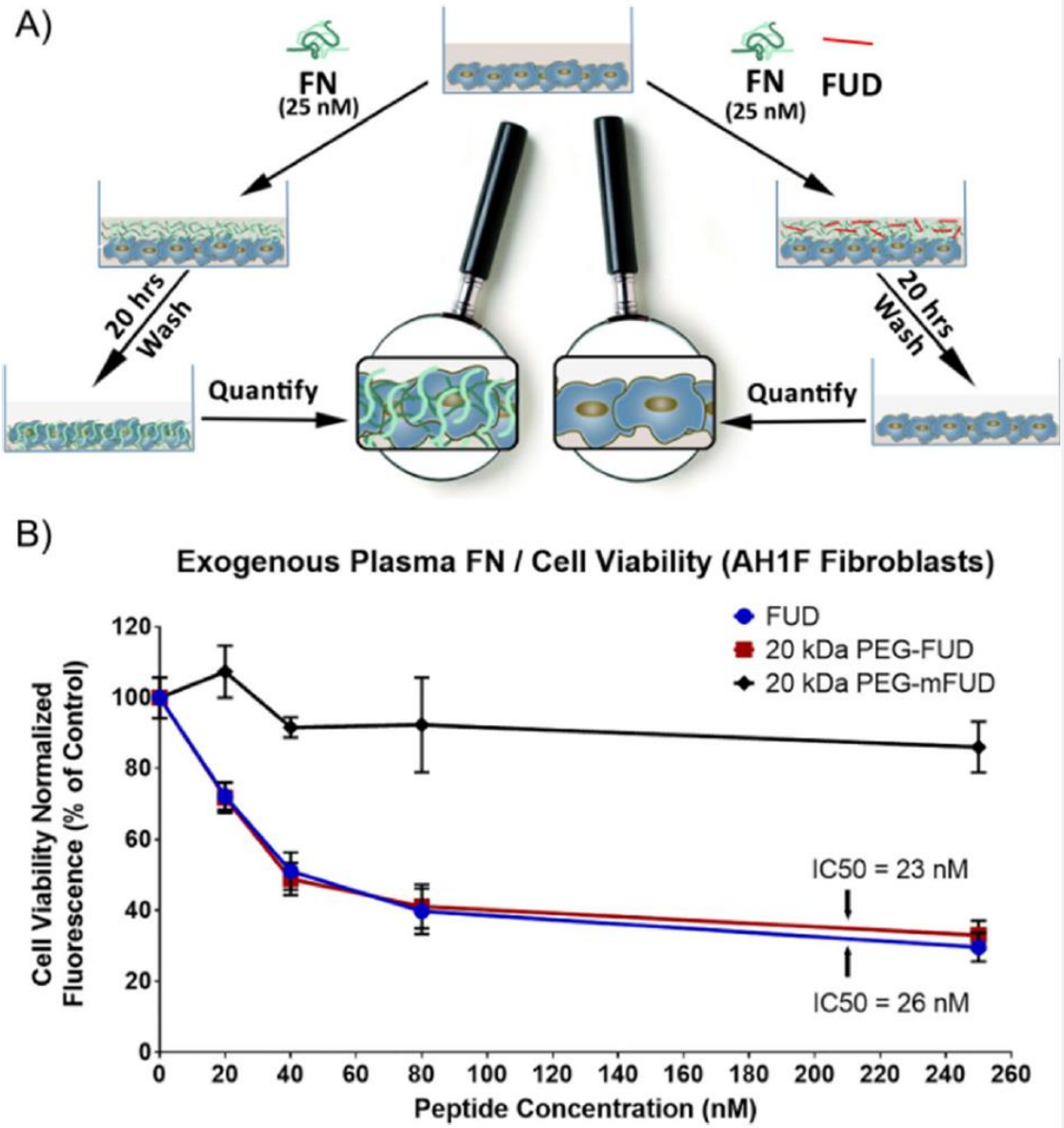
Matrix Assembly in vitro Assay (MAA)



Matrix Assembly in vitro Assay (MAA) demonstrating inhibitory potency of FUD and 20 kDa PEG-FUD conjugates, and the 20 kDa PEG-mFUD control peptide. (a) Schematic representation of assay methodology. Human foreskin fibroblasts (AH1F) are grown in the presence of exogenous Alexa 488-labeled human plasma FN and in the presence or absence of an inhibitor. (b) Results of a MAA experiment comparing 20 kDa PEG-FUD to FUD and showing parity of inhibitory potency. Extraction of IC50 values yielded 26 nM and 23 nM for FUD and 20 kDa PEG-FUD, respectively. For each data point, n=4.