Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.


# Supplementary

## A. An-FD influence on PPP in varied $Ca^{2+}$ concentrations and on purified fibrin polymerization by TEG

The effect of the An-FD peptide at 1 mM concentration on PPP was evaluated under different added $Ca^{2+}$ concentrations in attempt to compensate any competition over $Ca^{2+}$ ions with $Ca^{2+}$ dependent clotting factors. Samples were prepared as described in Experimental section of main text. Observed trends showed no advantage of increasing $Ca^{2+}$ concentration above 255 mM, indicating an inhibitory effect which could not be fully explained by competition over $Ca^{2+}$.

As An-FD showed inhibition of clot formation of PPP for both reduced clot rate formation and clot final strength (α and MA TEG parameters, correspondingly), results indicated interference with fibrin polymerization. Hence, the direct effect of An-FD on fibrin polymerization from purified protein solutions was evaluated. Solutions of purified fibrinogen and thrombin (Hyphen BioMed, Neuville-sur-Oise, France) at physiologically relevant concentrations were mixed with 11 mM final $Ca^{2+}$ concentration and An-FD solution or TBS (as control) and analyzed by TEG. Reduced polymerization rate (green curve) indicates the peptide's interference with fibrin polymerization which could be a result of the peptide's interaction with thrombin, fibrinogen or fibrin monomers.

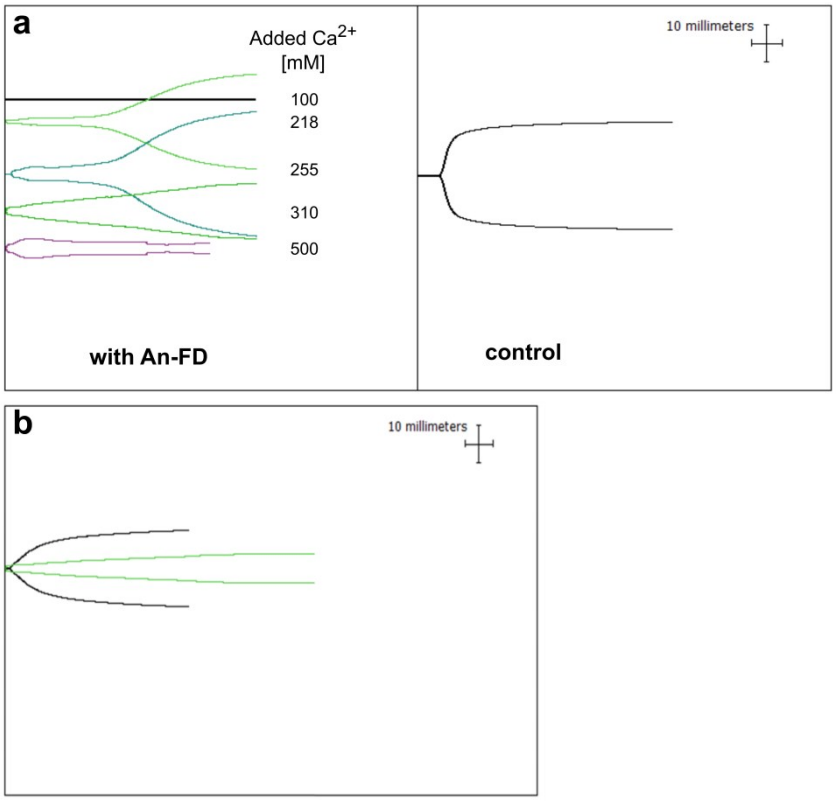


**Figure A: Representative TEG tracing illustrating the influence of 1 mM of An-FD on (a) pooled PPP supplemented with variable $Ca^{2+}$ concentrations (left) vs. standard 200 mM $Ca^{2+}$ supplemented PPP as control (right) and (b) fibrin polymerization achieved by reaction of purified thrombin (0.5 U/mL) and fibrinogen (5 mg/mL) supplemented with either peptide (green) or TBS (black) as control.**

## B. Peptide solutions show minimal hemolytic effect

The hemolytic effect of the peptides was tested at micromolar concentration, in order to model the effect of peptide dissolved out of hydrogels on red blood cells (RBCs). Fresh blood from four different animals (50 mL x4) was centrifuged at 3,044 g for 30 min at 4 °C followed by pipetting 2.5 mL from the bottom RBC phase of each tube and re-suspending collectively in 20 mL PBS (pH 7.4). Cells were washed with equal volume of PBS 3 times and finally diluted at 1:10 ratio.

**Hemolysis test:** samples (1 mL) comprising of 250 μL RBCs suspension, 500 μL PBS and 250 μL peptide solutions (6.4-0.008 mM in DIW), or DIW, PBS and 0.1% Triton X-100 as controls, were mixed and incubated at 37 °C for 45 minutes. Next, samples were centrifuged at 5000 g for 5 minutes at 4 °C and supernatant was sampled for absorbance (A) of hemoglobin at 540 nm. % hemolysis was calculated as follows:

$$\% \text{ hemolysis} = (A_{\text{sample}} - A_{\text{DIW cont.}})/(A_{\text{0.1\% Triton x-100 cont.}})$$

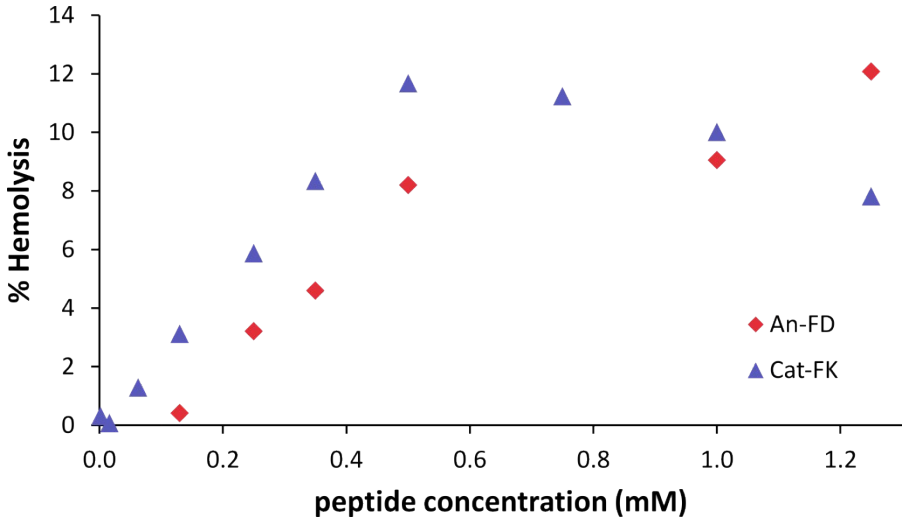


**Figure B: Hemolysis by peptide solution. Both An-FD and Cat-FK induce a slight hemolytic effect compared to 0.1% of Triton X-100, while Zwi-FDK showed a negligible hemolysis rate, lower than that measured with DIW, attributed to the salt content from peptide solution, relieving osmotic pressure. One point of low value for An-FD at 0.75 mM was discarded due to possible measurement error.**

## C. Supplementary TEG

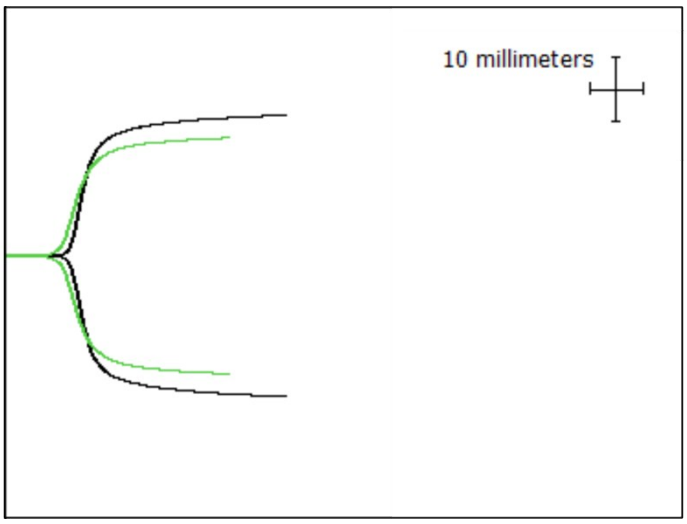


**Figure C: Representative electrograph of 1 mM Zwi-FDK influence on PPP clotting (green), showing reduction in R parameter versus TBS supplemented control (black), attributed to increased viscosity in the peptide mixed plasma.**

## D. Cat-FK's influence on blood

In a preliminary test, peptide solutions were mixed with PPP to assess any change in gross appearance. Cat-FK displayed a deleterious effect on plasma, causing phase separation (0.55 mM peptide in 2:3 diluted PPP, final concentrations). Separately, the same peptide seemed to sequester red blood cells (RBCs), forming peptide-cells clusters, probably due to electrostatic interactions between the cationic peptide and the negatively charged phospholipids of the RBCs membrane (0.25 mM peptide in RBCs suspension in PBS).

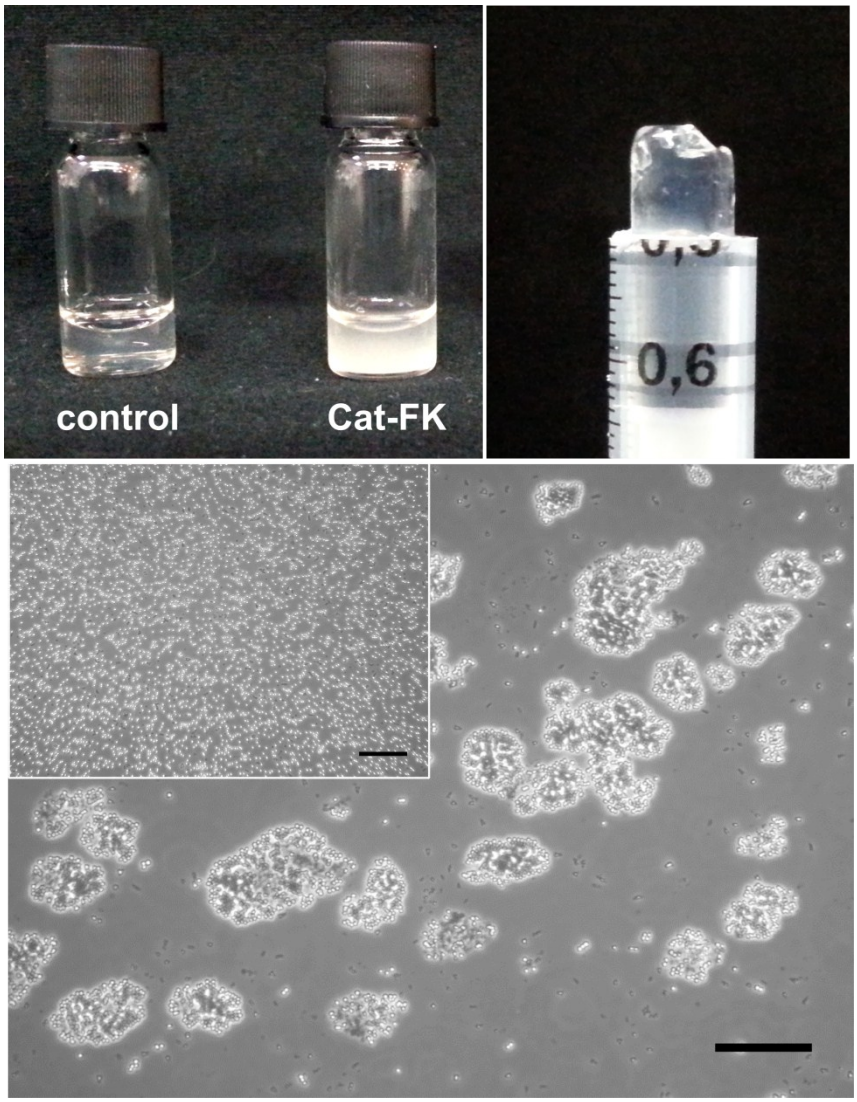


**Figure D: Top Left: Cat-FK solution mixed with PPP appears turbid, with visible sediments (0.55 mM peptide, 2:3 diluted plasma). Control is PPP supplemented with TBS, pH 7.4. An-FD and Zwi-FDK solutions were also mixed with PPP and showed no difference versus control. Top right: 4.2% w/v Cat-FK in 80 mM NaOH forms clear hydrogels of pH 9.8 Bottom: Light microscopy images obtained in phase mode show Cat-FK (0.25mM) sequestering RBCs from their suspension in PBS. Inset: control RBCs suspension in PBS. Bar=0.5 mm.**

## E. Peptide hydrogels influence on PPP clotting by TEG

Plasmas were assayed by normal TEG analysis after 3 minutes of incubation (37 °C) in contact with 4% w/v peptide hydrogels (see section 2.4.1 of Experimental in main text).

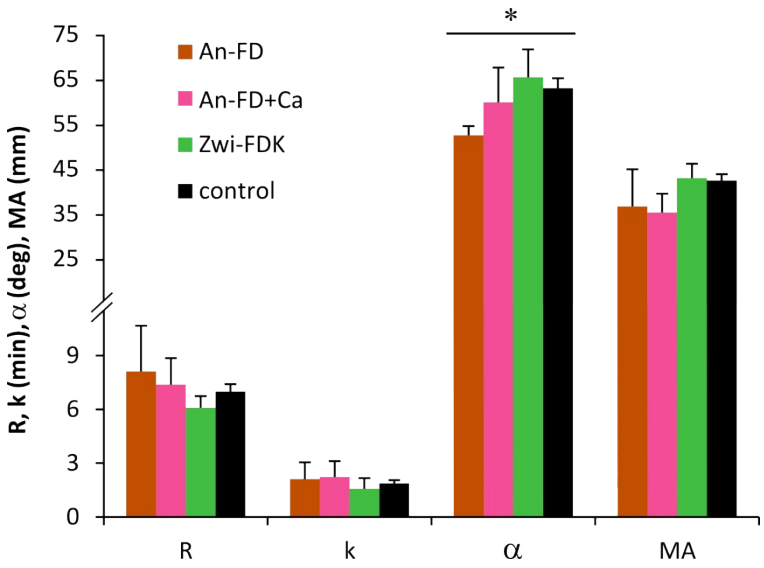


**Figure E: Clotting parameters of PPPs that were exposed for 3 min to 4% w/v peptide hydrogels (and to plastic Eppendorf tube as control) show moderate trends. Significance estimated vs. control samples by T test (*p<0.05, **p<0.01, ***p<0.001, n=6).**

## F. An-FD Hydrogel dissolution into $Ca^{2+}$ supplemented buffer (TBS-Ca) and rheology testing

The dissolution of peptide hydrogels was tested in TBS to allow for measurement of spectral absorbance of the Phe ring, to validate rates quantified otherwise (by the fluorescence of Rhodamin B labeled peptide, brought in the main text).

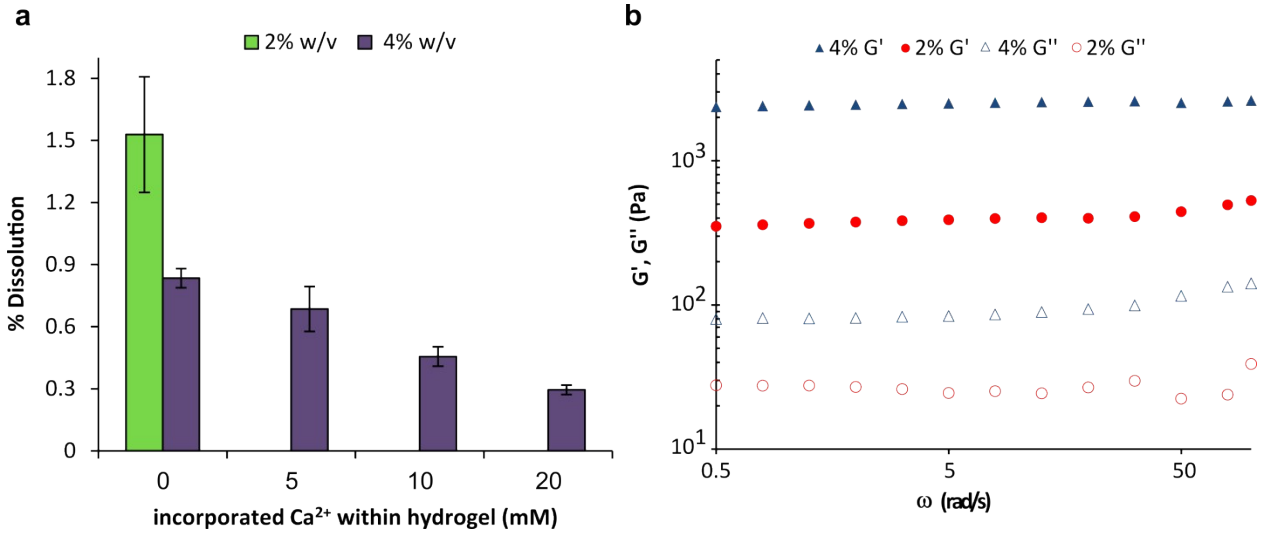


**Figure F: (a) Hydrogels dissolution by peptide released from An-FD hydrogels into TBS-Ca (2.5 mM $Ca^{2+}$) as a function of hydrogel incorporated $Ca^{2+}$ and peptide concentration. Peptide amount is normalized to initial peptide concentration in hydrogel. Note similar molar peptide concentration dissolved off 2% and 4% w/v hydrogels (0.19±0.03 versus 0.20±0.01 mM, correspondingly). (b) 2% and 4% w/v An-FD hydrogels storage (G') and loss (G'') moduli.**

## G. Peptides effect on clotting proteins

Both Cat-FK and ScAn-FD exhibit spectral ellipticities at 220 nm and 205 nm attributed to random conformation (similar to PolyProline I absorbance) [1] and a positive absorbance band at 200 nm which may be attributed to Phe---Phe stacking interactions. [2]

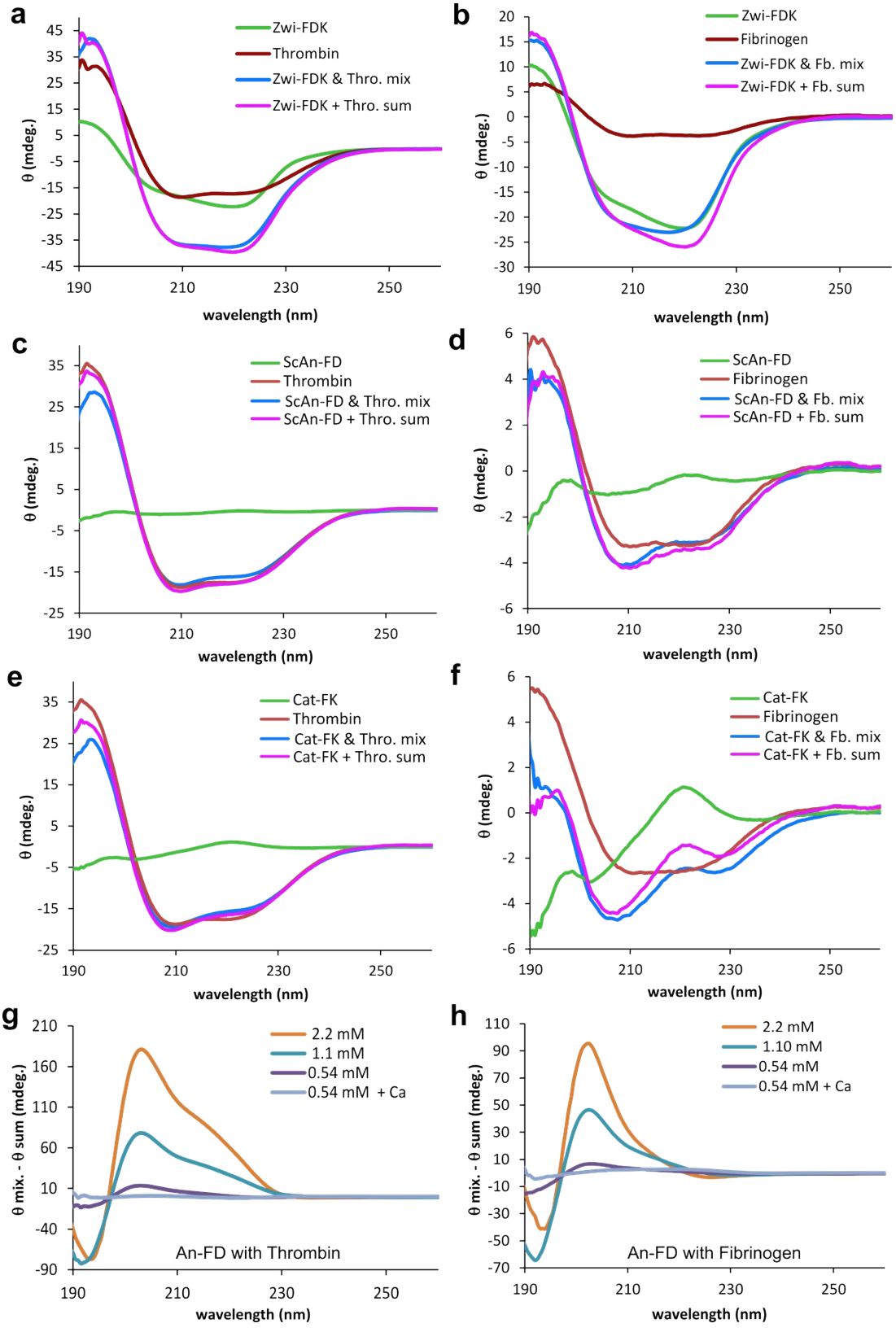

**Figure G: CD spectra of Zwi-FDK (a-b), ScAn-FD (c-d), Cat-FK (e-f) and protein solutions, comparing ellipticities of mixtures to the theoretical sum of the individual components as an indication of peptide-protein interactions. 0.54 mM peptide (green), 1 U/mL thrombin and 1 mg/mL fibrinogen (dark red), measured spectra of peptide and protein mixture (blue) and the theoretical sum of the mixture spectra (magenta).. Zwi-FDK spectra displayed two minima at 202 nm and 222 nm with the latter attributed to α-helical structure (in DIW this peptide shows random and β-sheet structures). (g-h) Difference plots representing An-FD mixed with (g) 1 U/mL thrombin or (h) 1mg/mL fibrinogen spectra subtracted of the spectra of the unmixed components at varying peptide concentrations and 2.5 mM $CaCl_2$ (where Ca is specified).**

## H. Peptide–protein interactions visible by aggregate formation

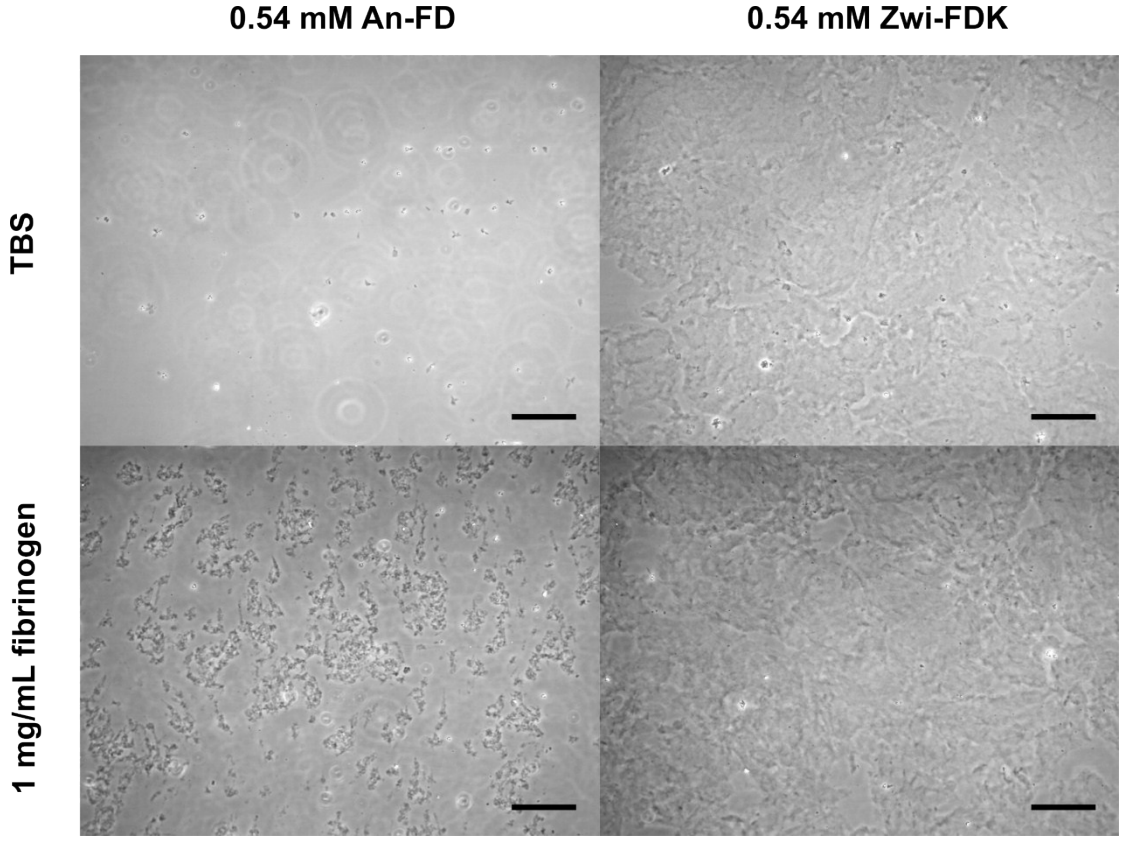


**Figure H: Light microscopy images obtained in phase mode of fibrinogen with 0.54 mM An-FD with show fine aggregates, and with 0.54 mM Zwi-FDK showing extended sheets, which are also visible in this peptide pure solution. Bar 0.5 mm.**

# I. Bleeding spleen model

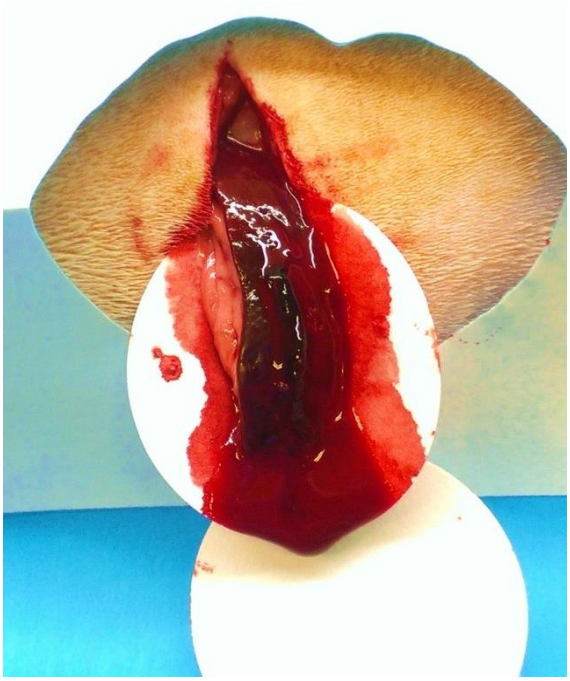

**Figure I: Representative images of naive 4% An-FD hydrogel injection into bleeding rat's spleen laceration wound. 1.5 cm long cut was made along the midline on the top apparent surface of the spleen before injecting the hydrogel. Notice the pancreas attached to the bottom of the spleen, visible as pink tissue. Notice the hydrogel as a dark band within the wound, conveying the color of the inner tissue.**

# J. Peptides zeta potential

Zeta potential values of peptide solutions 0.54 mM in 1:0.57 diluted TBS were measured by Zetasizer (Zetasizer Nano ZS, Malvern, Worcestershire, UK) :

Table J: Peptide solutions zeta potentials (mV)*

| Zwi-FDFK | An-FD | ScAn-FD | Cat-FK |
|---|---|---|---|
| 1.20±0.68 | -13.13±0.85 | -11.68±0.84 | 8.06±0.87 |

* Each sample averaged over 3 measurements and repeated in triplicates.

## K. Equimolar mixture of An-FD and Cat-FK show microscopic sheets structures

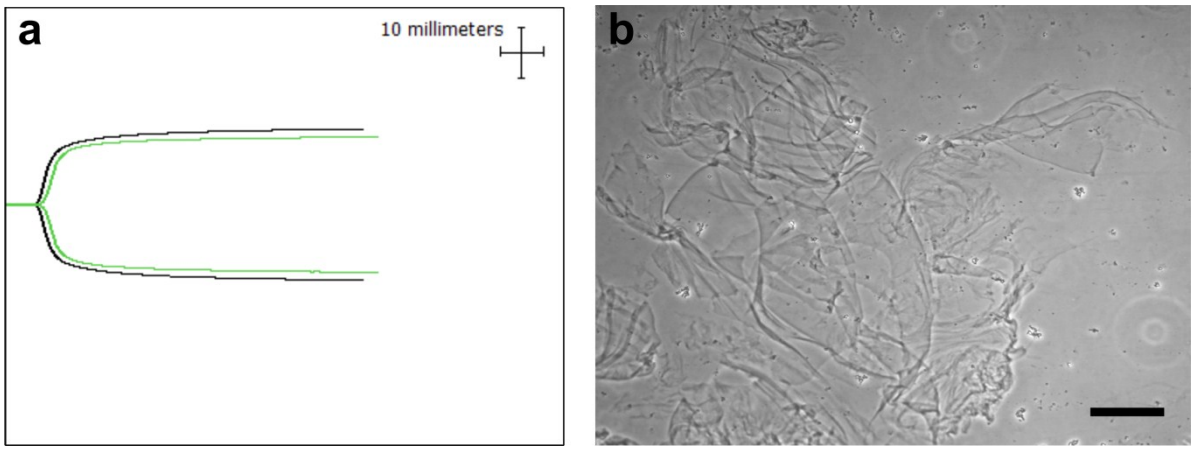


**Figure K: (a) Representative thromboelsatogram of 0.5 mM An-FD pre-mixed with 0.5 mM Cat-FK (total 1 mM, green) showing neglectable influence on PPP clotting versus TBS supplemented control (black), supported by co-assembly of the two peptides, as visible in (b) Light microscopy images obtained in phase mode show large sheet-like structures obtained by mixing of 0.27% w/v An-FD with 0.28% w/v Cat-FK 3 day old solutions (total 0.54% w/v, ~3.3 mM peptide). Solutions of each peptide lacked visible structures. Bar = 0.5 mm.**

References:

1. He L, Navarro AE, Shi Z, Kallenbach NR. End effects influence short model peptide conformation. *J Am Chem Soc*. 2012;134(3):1571-1576.

2. Shlomo Z, Vinod T, Jelinek R, Rapaport H. Stacking interactions by two phe side chains stabilize and orient assemblies of even the minimal amphiphilic β-sheet motif. *Chemical Communications*. 2015.