Electronic Supplementary Material (ESI) for Nanoscale Advances.

Supplementary Information

A Self-assembling Peptide Hydrogel for Ultrarapid 3D Bioassays

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1. Peptide synthesis and characterization

Materials

Rink amide resin, HMPB resin, *N*-α-Fmoc-L-amino acids and building blocks used during chain assembly were purchased from Iris Biotech GmbH (Marktredwitz, Germany). Ethyl cyanoglyoxylate-2-oxime (Oxyma) was purchased from Novabiochem (Darmstadt, Germany), *N,N'*-dimethylformamide (DMF) and trifluoroacetic acid (TFA) were from Carlo Erba (Rodano, Italy). N,N'-diisopropylcarbodiimide (DIC), dichloromethane (DCM) and all other organic reagents and solvents, unless stated otherwise, were purchased in high purity from Sigma-Aldrich (Steinheim, Germany). All solvents for solid-phase peptide synthesis (SPPS) were used without further purification. HPLC grade acetonitrile (ACN) and ultrapure 18.2 Ω water (Millipore-MilliQ) were used for the preparation of all buffers for liquid chromatography. The chromatographic columns were from Phenomenex (Torrance CA, USA). HPLC eluent A: 97.5% H_2O, 2.5% ACN, 0.7%TFA; HPLC eluent B: 30% H_2O, 70% ACN, 0.7%TFA

Peptide Synthesis: General Procedures

Resin loading

Resin (0.5 mmol/g loading) was swollen in CH_2Cl_2 for 30 min then washed with DMF (3 × 3 mL). A solution of entering Fmoc- amino acid, HBTU and DIEA (1:1:2, 5 eq over resin loading) in NMP (3 mL) was added and the resin shaken at rt for 4 h. For HMPB resin only 5% DMAP was added to the mixture. The resin was washed with DMF (2 × 3 mL) and capping was performed by treatment with acetic anhydride/ DIEA in DCM (1 x 30 min). The resin was then washed with DMF (2 × 3 mL), CH_2Cl_2 (2 × 3 mL), and DMF (2 × 3 mL). The resin was subsequently submitted to fully automated iterative peptide assembly (Fmoc-SPPS).

Peptide Assembly via Iterative Fully Automated Microwave Assisted SPPS

Peptides were assembled by stepwise microwave-assisted Fmoc-SPPS on a Biotage ALSTRA Initiator+ peptide synthesizer, operating in a 0.1 mmol scale. Activation of entering Fmoc-protected amino acids (0.3M solution in DMF) was performed using 0.5M Oxyma in DMF / 0.5M DIC in DMF (1:1:1 molar ratio), with a 5 equivalent excess over the initial resin loading. Coupling steps were performed for 7 minutes at 75°C. Fmoc- deprotection steps were performed by treatment with a 20% piperidine solution in DMF at room temperature (1 x 10 min). Following each coupling or deprotection step, peptidyl-resin was washed with DMF (4 x 3.5 mL). Upon complete chain assembly, resin was washed with DCM (5 x 3.5 mL) and gently dried under a nitrogen flow.

Cleavage from the Resin

Resin-bound peptide was treated with an ice-cold TFA, TIS, water, thioanisole mixture (90:5:2.5:2.5 v/v/v/v, 4mL). After gently shaking the resin for 2 hours at room temperature, the resin was filtered and washed with neat TFA (2 x 4 mL). The combined cleavage solutions were worked-up as indicated below.

Work-up and Purification

Cleavage mixture was concentrated under nitrogen stream and then added dropwise to ice-cold diethyl ether (40 mL) to precipitate the crude peptide. The crude peptide was collected by centrifugation and washed with further cold diethyl ether to remove scavengers. Residual diethyl ether was removed by a gentle nitrogen flow and the crude peptide was purified by RP-HPLC. Collected peptide was quantified by UV spectroscopy, diluted to 0.05 mM concentration and aliquoted. Peptide aliquots were stored at -80°C, then lyophilized before use.

Synthesis of Cyanine-labelled peptides

Cysteine-bearing peptides were conjugated to bifunctional MAL-Cy3/MAL-Cy5 (Lumiprobe GmbH, Germany) as follows: peptide (1 eq.) was dissolved in phosphate buffer (Na_2HPO_4 0.4M, pH 7.8). The resulting solution was ice-cooled and mixed with MAL-Cy3/MAL-Cy5 solution (1.2 eq., 50:50 acetonitrile/water mixture). The reaction mixture was left to react for under gentle shaking until full reagents conversion (RP-HPLC monitoring). Upon reaction completion, conjugation products were isolated by preparative RP-HPLC and lyophilized.

Synthesis of YF-Q11 conjugates via CuAAC

YF-Q11-N_3 (1eq.) was dissolved in water. The resulting solution was mixed with a solution of propargyl-bearing conjugation partner (1.1 eq.), then $CuSO_4$ (2eq.) and ascorbic acid (5eq.) were added. The reaction mixture was let to react for under gentle shaking until full reagents conversion (RP-HPLC monitoring). Upon reaction completion, conjugation products were isolated by preparative RP-HPLC and lyophilized.

RP-HPLC analysis and purification

Analytical RP-HPLC was performed on a Shimadzu Prominence HPLC (Shimadzu) using a Shimadzu Shimpack GWS C18 column (5 micron, 4.6 mm i.d. x 150 mm). Analytes were eluted using a binary gradient of mobile phase A (100% water, 0.1% trifluoroacetic acid) and mobile phase B (30% water, 70% acetonitrile, 0.1% trifluoroacetic) using the following chromatographic method: 10% B to 100% B in 14 min; flow rate, 1 ml/min.

Preparative RP-HPLC was performed on a Tri Rotar-VI HPLC system (JASCO) using a

Phenomenex Jupiter C18 column (10 micron, 21.2 mm i.d. x 250 mm) using the following chromatographic method: 0% B to 90% B in 45 min; flow rate, 14 ml/min. Pure RP-HPLC fractions (>95%) were combined and lyophilized.

Electro-spray ionization mass spectrometry (ESI-MS)

Electro-spray ionization mass spectrometry (ESI-MS) was performed using a Bruker Esquire 3000+ instrument equipped with an electro-spray ionization source and a quadrupole ion trap detector (QITD).

2. Microarrays: General Procedures

Reagents

Goat anti-human IgG labeled by Cy3 and Alexa Fluor® 647-conjugated Streptavidin were obtained from Jackson Immunoresearch (West Grove, PA, USA). Monoclonal antibody to Pk (V5) Epitope Tag (GKPIPNPLLGLDST) was purchased from OriGene Technologies Inc. (Rockville, MD, USA). Goat polyclonal anti-Alpha-lactalbumin antibody was obtained from GeneTex (Irvine, CA, USA). V5 antibody and Alpha-lactalbumin antibody were labelled with Cy3 and Cy5 (Lumiprobe GmbH, Hannover, Germany) *in-house* according to the manufacturer's instructions. PMMA slides were from microfluidic ChipShop GmbH (Jena, Deutschland). A panel of positive ZIKV serum samples was purchased from Boca Biolistics (Pompano Beach, FL). Samples were proved positive by MAC Elisa test performed by the purchaser and confirmed positive for anti-Zika IgG by an NS1 based ELISA. Human sera from healthy donors used in the study were obtained from voluntaries, which donated blood for transfusion purposes and their use was permitted for research purposes only. Both donor and recipient's identities are protected by the privacy laws. The purpose of production of blood products is only of the donor's free will and with the latter's consent. Demographic details in the Tables S4

Spotting procedure

PMMA slides (Microfluidic ChipShop, Jena, Germany) were pre-treated with a 1% w/v Milk Powder solution in PBS for 1 hour at room temperature, washed with distilled water and dried under nitrogen stream. Different concentrations of YF-Q11 gel (500 μM, 250 μM, 100 μM, 50 μM, 25 μM) were incubated at 40°C for two hours and then spotted using a noncontact microarray spotter (Scienion sciFLEXARRAYER S12) with a 80μM noozle, 20 droplets are deposited for each spot (approximately volume 8 nL). Immediately after spotting, all slides were stored for 2 hours in a sealed chamber, saturated with sodium chloride. Before use slides were briefly rinsed with water, dried under a nitrogen stream and scanned to evaluate initial fluorescence intensity.

Fluorescence analysis

In all microarrays experiments, dry slides were scanned by a TECAN Power Scanner using the Cy3 (Ex 550nm; Em 570 nm) and Cy5 (Ex 650 nm; Em 670 nm) channels and analysed according to conventional microarray analysis protocols and instrumentation with the aid of the analysis software ScanArray Express from PerkinElmer. The software provides the mean fluorescence intensity of each spot corrected for spot-specific background. The RFI data reported in the paper were averaged from replicated spots; the standard deviation of each measurement is indicated by the error bar reported in graphs. Different scanning protocols in terms of laser power and Photomultiplier (PMT) gain were used for the two channels, depending on the experiment, to avoid signal saturations for quantification purposes.

3. Hydrogel tests

Hydrogel formation

Freshly lyophilized YF-Q11 aliquots were dissolved in milliQ water to 500□M concentration. Resulting solution was then sonicated for 5 min, diluted to desired concentration and then incubated at 40°C for 2 hours. The resulting soft hydrogels were directly used for analysis or microarrays.

Droplets volume analysis

A YF-Q11 hydrogel droplet (25 μM) was deposited on a PMMA slide, let spontaneously dehydrate, briefly rinsed in water to remove any unbound material and let dehydrate again. The gel droplet was then rehydrated by a brief immersion in water and then monitored by a CAM 200 instrument (KSV Ltd), which utilizes video capture and subsequent image analysis to calculate the droplet volume for approximately 100 seconds (Figure S7). In this time frame, the volume progressively decreases from 1 to 0.5 μL; further volume decrease is no longer observable by the instrument. Drying and rehydration cycles were repeated for three times.

Stability

Different concentrations of YF-Q11 (500 μM, 250 μM, 100 μM, 50 μM, 25 μM) were added with Cy3-labelled Q11 at the concentration of 0.1 μM and spotted on the PMMA slides as previously described. Stability was assessed by evaluating residual Cy3 fluorescence after washing steps (2, 5, 10, 30, 60 minutes) in a washing buffer solution (0.05 M Tris/HCl pH 9, 0.25 M NaCl, 0.05% v/v Tween 20), a brief rinse with water and drying under a nitrogen stream. Scanner was set at 10% laser power and 100% PMT gain. The time 0 intensity was not considered as initial fluorescence in the derived graphs as the RFI was at saturation, therefore not quantifiable in the scanning conditions used for assessment of biomolecule residual fluorescence. Furthermore, as described in section 2.1, within the first minute of incubation an initial loss of hydrogel outer shell was observed that would have flawed data quantification.

Biomolecules diffusion

YF-Q11 (500 μM, 250 μM, 100 μM, 50 μM, 25 μM) gels were mixed with 1μM of Cy5-labelled Antibody anti-α-lactalbumine, AlexaFluor 647-conjugated Streptavidin and Cy5-labelled V5. For each concentration an empty control gel was also spotted. The slides were washed for 2, 5, 10, 30 and 60 minutes in the washing buffer solution, then briefly rinsed with water, dried under nitrogen stream and scanned to estimate the biomolecules diffusion by detection of the Cy5 residual fluorescence of 6 replicated spots (Scanner was set at 10% laser power and 30% PMT gain).

Symmetrical antibody-peptide recognition assays

a) Peptide-functionalized hydrogels probed with antibodies:

Covalent conjugates of V5 and LAC peptides with YF-Q11 were obtained via a click chemistry approach, and added at a 10% ratio in the YF-Q11 hydrogel matrix during the pre-printing step at 40°C. The epitope-functionalized hydrogel spots were then incubated for 5 minutes with their specific fluorescently labelled antibodies (Cy3-anti-V5 and Cy5-anti-LAC) at the concentration of 1μg/mL in a 1:1 a mixture in incubation buffer (0.05 M Tris/HCl pH 7.6, 0.15 M NaCl, 0.02% v/v Tween 20), washed for two minutes with washing buffer (0.05 M Tris/HCl pH 9, 0.25 M NaCl, 0.05% v/v Tween 20) and scanned for fluorescence evaluation of 3 replicated spots at 50% laser power and 200% PMT gain in the Cy3 channel and 50% laser power and 1000% PMT gain in the Cy5 channel.

b) Antibody-functionalized hydrogels probed with peptides:

A 250 μM YF-Q11 solution was used to individually entrap the anti-V5 and anti-LAC antibodies added in the pre-printing step at the concentration of 1μM. The spotted antibody-functionalized hydrogels were then incubated with fluorescently labelled-peptides (Cy3-V5 and Cy5 LAC) at the concentration of 5 μM in a 1:1 a mixture in incubation buffer for 5 minutes, washed with washing buffer for 2 minutes and scanned for fluorescence evaluation of 3 replicated spots at 10% laser power and 200% PMT gain in the Cy3 channel and 100% laser power and 50% PMT in the Cy5 channel.

Saturation kinetics for V5-Anti-V5 pair

PMMA slides were arrayed with YF-Q11 co-assembled with V5 peptide at 20% (total spotted peptide concentration 5 μM) and probed with a Cy3 labelled anti-V5 antibody (1 ng/mL) at different incubation times ranging from 1 to 10 minutes. Averaged fluorescence response of 8 gel spots is reported in Figure S9 (red line). The same experiment was run on a flat surface (blu line) by spotting 5 μM V5 peptide on polymer modified PMMA slides for covalent binding.

Optimization of V5 content into hydrogel

25 μM YF-Q11 hydrogels (8 replicated spots) containing an increasing amount of co-assembled V5 epitope (from 0% to 50%) were arrayed on PMMA slides. The microarrays were incubated for 5 minutes with different concentrations (from 0 ng/mL to 100 ng/mL) of anti-V5 IgG spiked into human serum diluted 1:100. Microarrays were then washed (2 min) and incubated with Cy3-anti mouse IgG (2 min) to reveal the binding of anti-V5 on its specific in-gel epitope. For all gels containing more than 1% V5 epitopes, signals for the corresponding antibody capturing at 5 ng/mL were clearly distinguishable from the blank (Figure S11). The functionalized spots containing 20% of V5 epitope provided the highest signal/noise ratio in the 5-50 ng/mL range of anti-V5 IgG and were selected for further immunoassays described in the main text.

Zika diagnostic assay

For the Zika diagnostic assay, serum samples from Zika positive patients and healthy controls were diluted 1:50 in incubation buffer (0.05 M Tris/HCl pH 7.6, 0.15 M NaCl, 0.02% v/v Tween 20). Sera were incubated over the gel array (6 replicated spots) for 5 minutes and then washed for 2 minutes in washing buffer, rinsed with water and dried under nitrogen stream. Then, slides were incubated for 2 minutes with 1 ug/mL anti-human IgG secondary antibody in incubation buffer followed by a washing of 2 minutes in PBS, then rinsed with water, dried and scanned at 50% laser power and 800% PMT gain.

4. Material characterization

Circular dichroism

CD experiments were carried out in deionized water (18.2 MΩ cm) in a 0.1 cm quartz cuvette, using a JASCO J1100 CD-spectrometer. Acquisitions were performed between 190 and 260 nm with a 0.1 nm data pitch, 1 nm bandwidth, 100 nm min−1 scanning speed and 1 s response time. All the spectra are an average of 5 scans and were corrected from a deionized water reference solution. Further details are provided in the SI.

Spectrofluorimetry

Fluorescence measurements were carried out using a Jasco FP-550 spectrofluorometer in 1 cm quartz cuvettes containing 0.5ml of solution. Samples were mixed with appropriate amounts of dyes immediately prior to the measurements. In a typical experiment 1 uM of dyes were added to a solution of YF-Q11 and spectra were recorded at regular intervals. The temperature was maintained by a thermo-stated Peltier cell holder.

ATR-FTIR

Infrared spectra of YFQ11 were recorded using a Nicolet Nexus FTIR spectrometer equipment with U-ATR device from 400 to 4,000 cm−1. The spectra were corrected for the baseline. For each sample, spectra were collected (128 scans) using solid-state substrate beam splitter.

AFM measurements

AFM measurements were done with a NT-MDT SMENA head in non contact mode using NSG30 cantilevers at 0.8 - 1 Hz and about 50 nm oscillation amplitude. Images have been planarized using GWyddion v2.49 by subtracting line-by-line a 2nd order polinomial and aligning rows using the median filter [47].

Rheometry

A KINEXUS Pro+ rheometer (MalvernPanalytical, UK) was used to measure viscoelastic properties of the gel. Gels were pre-formed and directly transferred on the bottom rheometer plate. The upper geometry Cone 1°/60mm, was lowered until it was in conformal contact with the top surface of the hydrogel, corresponding to gap distances of 1.0-1.5 mm, and then lowered to the final working position (0,07 mm) monitoring the normal force the gel react with, when pressure is applied to it. Measures were performed at 25°C. Further information are reported in the Supporting Information.

Confocal Raman imaging

Raman images of hydrogel were acquired by using the InVia Reflex confocal Raman microscope (Renishaw plc, Wotton-under-Edge, UK). A hydrogel solution (250 μM) was mixed with pig-IgG (12 μM) previously labeled with malachite green isothiocyanate (M689, ThermoFisher scientific, MA , USA). The hydrogel-antibody mixture and the empty hydrogel were then spotted by a piezoelectric spotter onto Raman-compatible CaF2 discs (Crystran, UK) and then immersed in water before the Raman analysis. The Raman study was performed using a 785nm excitation laser with 100% power, a 1200 l/mm grating and a LUMPLFL 60X Water objective (Olympus). At each z-position (from 0 to 18 μm, with z-step of 3 be μm) a Raman rectangle mapping was carried out by acquiring 1248 (52 x 24) single spectra applying and acquisition time of 2 sec x 2 and using a raster scanning mode with 18 μm step-size. For the data analysis the WiRE 5.0 software (Renishaw plc, UK) was used. Data pre-processing only involved cosmic-rays removal using both nearest-neighboring and width-features algorithms. Raman images at each focus plan were obtained by direct classical least squares (DCLS) component using as single reference a previously collected spectrum of malachite labeled-antibody. 3D volume rendering was created by ImageJ 1.51 software (NIH, USA), using the volume viewer plugin.

5. Supplementary Figures

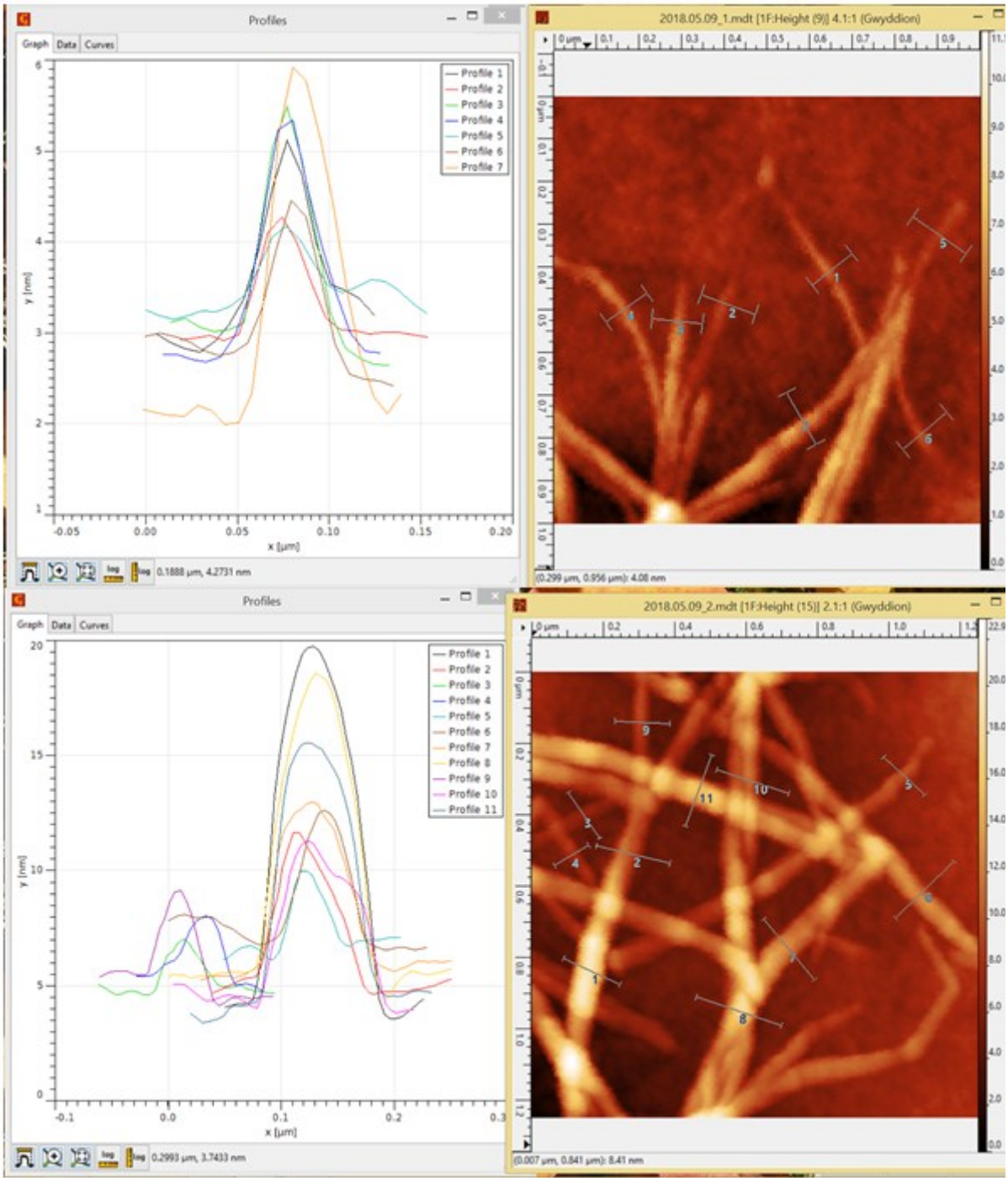

Figure S1. AFM measurements (height, nm) of smallest detectable fibers (upper panel) and thicker, bundled fibers (lower panel).

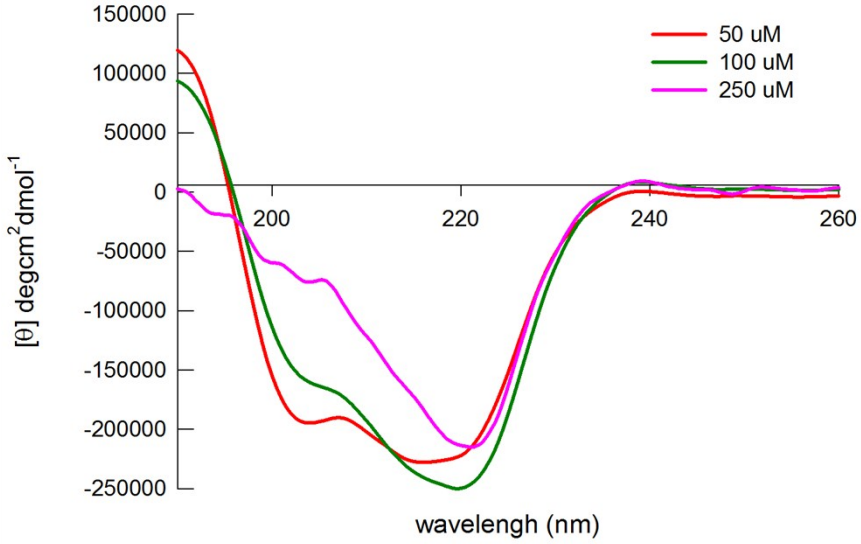

Figure S2. CD spectra of YF-Q11 at different concentration recorded after incubation for 2h at 40°C

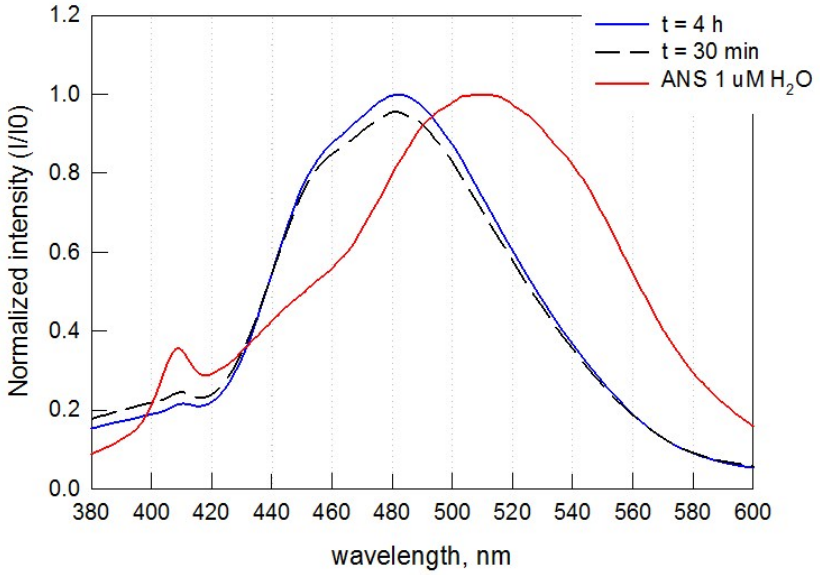

Figure S3. Plot of normalized fluorescence spectra of ANS in water (red line) and in the presence of YFQ11 at different times (dotted line: 30min, blue line: 4h). ANS shows a very weak fluorescent emission band in water, centered at 510 nm (red line). In the presence of YFQ11 (250 □M) the fluorescence emission rapidly increases and a blue shift in the emission band is observed, pointing out to an increased hydrophobic microenvironments sensed by the fluorescent probe.

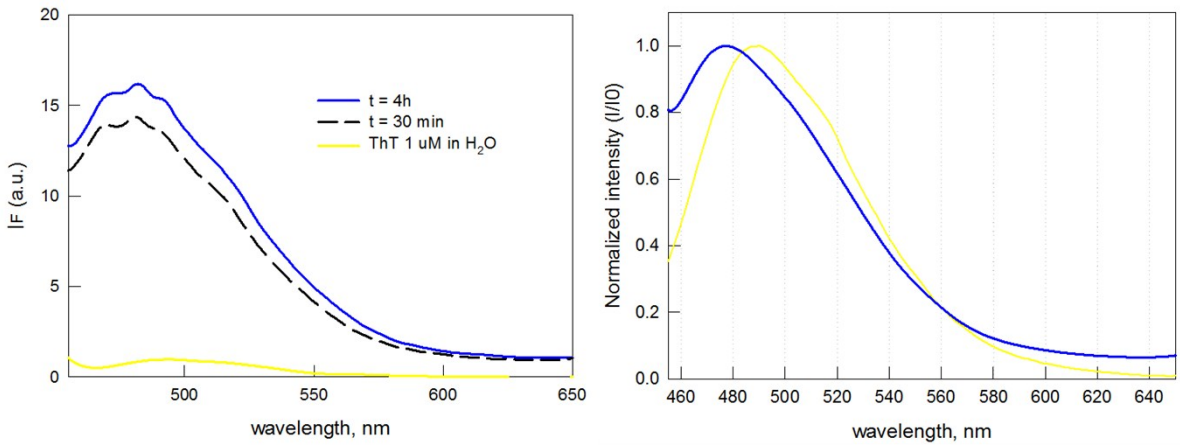

Figure S4. a) Fluorescence spectra of thioflavine (1 μM) in water (yellow line) and in the presence of 250 μM YFQ11 (blue and black lines) at different times (λex = 440 nm). b) Normalized fluorescence spectra of ThT in water (yellow line) and in the presence of YFQ11 (blue line).

Figure S5. YF-Q11 hydrogel spotted microarray slide. Dry spots (left panel) instantaneously rehydrates upon immersion in water (right panel).

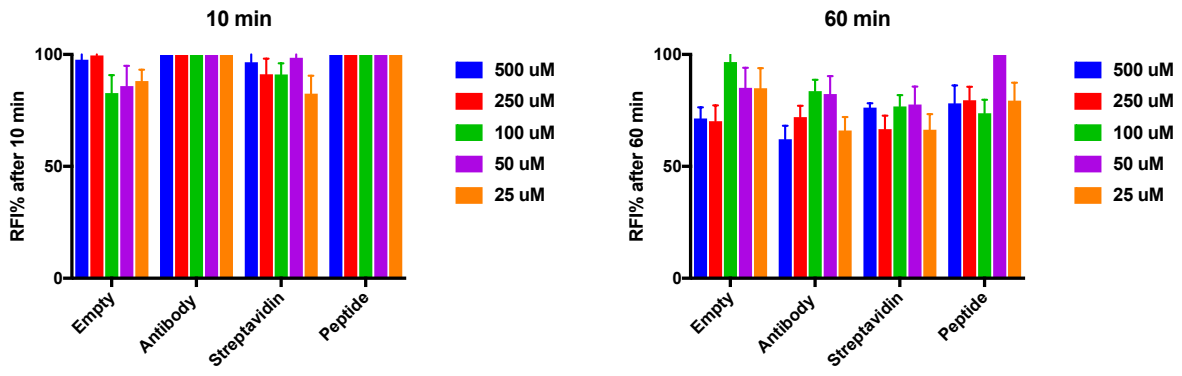

Figure S6. Hydrogel spot stability assessment. Residual Cy3 fluorescence detected on slides for gel concentrations ranging from 500 to 25 µM after 10 min (left panel) and 60 minutes (right panel). All fluorescence scan were simultaneously acquired. Fluorescence detection by 10% laser power and 100% Photomultiplier Gain, parameters set to monitor simultaneously both fluorescence channels and all added biomolecules. The whole range of concentrations (from 500 µM to 25 µM) showed a good stability over time with at least 90% of residual fluorescence detected after 10 minutes and 70% after 60 minutes. Empty gel's behaviour was similar to that of gels added with other components, suggesting that gel stability is not affected when YF-Q11 is mixed with biomolecules prior to spotting.

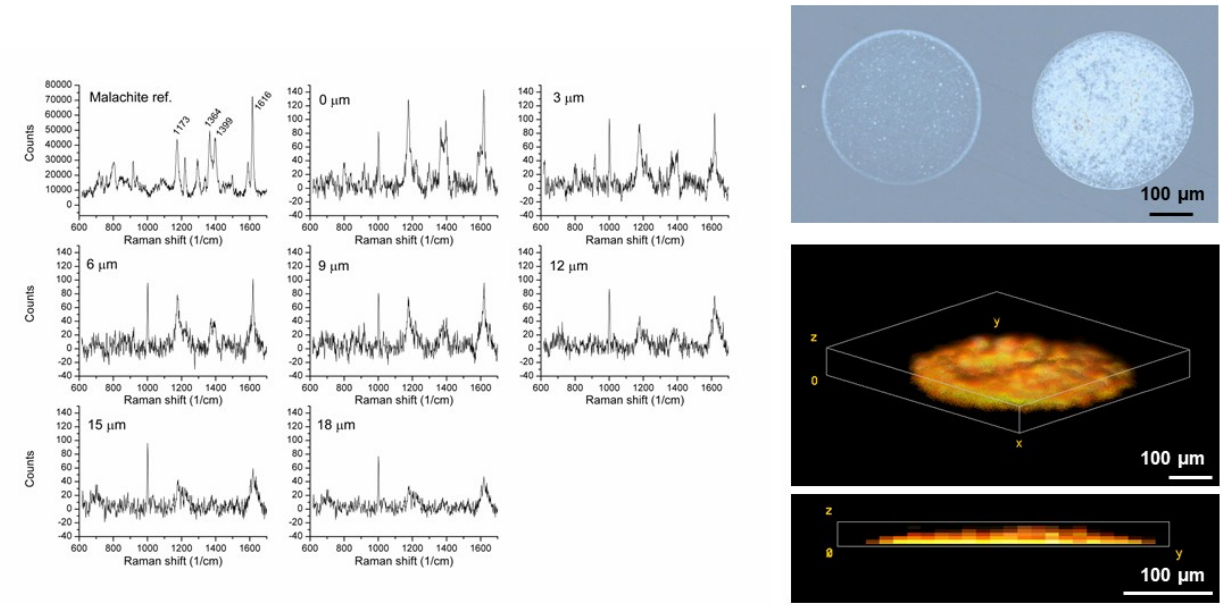

Figure S7. Single Raman spectra of malachite green (acqueous solution, used as reference for DCLS component analysis) and of random points inside the hydrogel microdrops containing malachite-labeled Ab, mapped at different height (left panel). Each spectrum is the average of five spectra selected from different position inside the microdroplets. In the first panel the wavenumbers assignations are reported for the main malachite green Raman signals. Bright field (BF) image captured before the Raman mapping (right) and two different projection views of 3D volume rendering images derived by merging the seven stacked images.

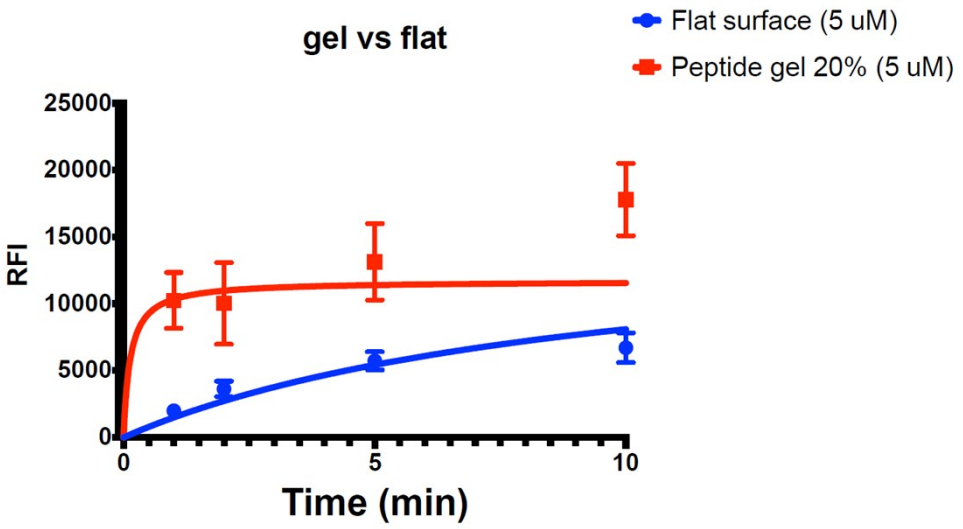

Figure S8. Fluorescence signal of the V5-antibody pair interaction at different incubation time on hydrogel microarray (red line) and flat surface (blue line). 5 µM V5 peptide was spotted on the flat PMMA slide or added to the hydrogel droplets by co-assembly; slides were incubated with 1 ng/mL antibody. Fluorescence detection by 50% laser power and 80% Photomultiplier Gain

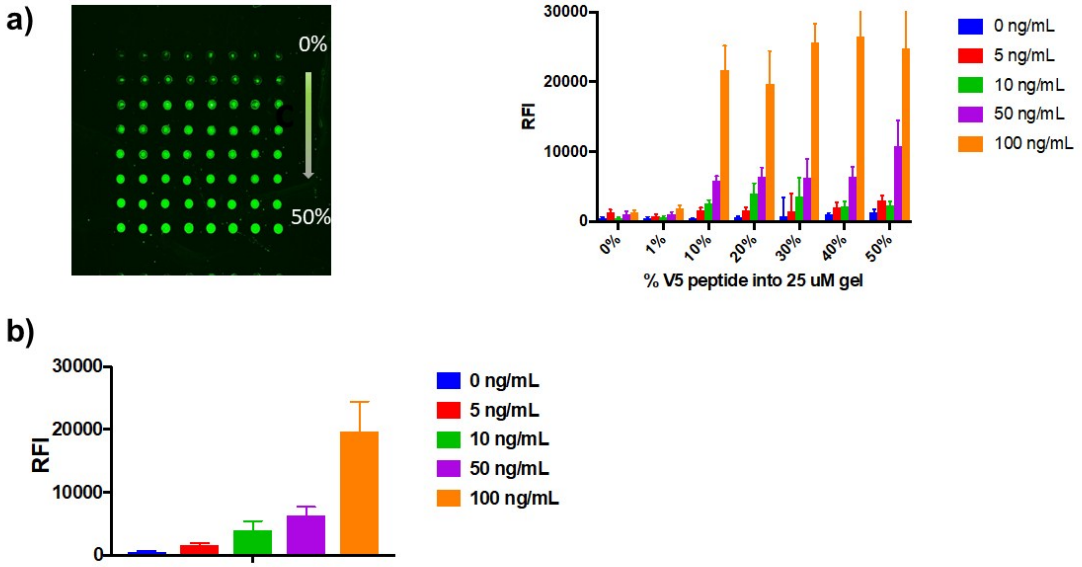

Figure S9. Optimization of V5 epitope content in hydrogel spots. Increasing amount (from 0% to 50%) of V5 peptide were co-assembled in the hydrogel and spotted on PMMA slides.
The microarray was then incubated with Anti-V5 IgG from 0 ng/mL to 100 ng/mL spiked into 1:100 human serum followed by incubation with fluorescent secondary antibodies. Fluorescence scanning shows that all gels containing more than 1% V5 epitopes are clearly distinguishable from the blank. Fluorescence detection by 50% laser power and 80% Photomultiplier Gain

1. Tables

Table S1. Peptide list

Code	Sequence
YF-Q11	Ac-YFQQKFQFQFEQQ-NH2
YF-Q11-Cy3	Ac-C(Cy3)-YFQQKFQFQFEQQ-NH2
V5-Cy3	C(Cy3)G-GKPIPNPLLGLDST-COOH
LAC-Cy5	C(Cy5)G-EQLTKAEVFRELKDLKGYG-COOH
YF-Q11-N_3	Ac-K(N_3)-GG-YFQQKFQFQFEQQ-NH2
V5*	Prg-$(O2Oc)_2$-GKPIPNPLLGLDST-COOH
LAC**	Prg-$(O2Oc)_2$-EQLTKAEVFRELKDLKGYG-COOH
ZEp1	Prg-$(O_2Oc)_2$-VNELPHGWKAWGKSYFVRAAKT-COOH
YF-Q11-V5	Ac-X-GG-YFQQKFQFQFEQQ-NH2 X-$(O2Oc)_2$-GKPIPNPLLGLDST-COOH
YF-Q11-LAC	Ac-X-GG-YFQQKFQFQFEQQ-NH2 X-$(O2Oc)_2$- EQLTKAEVFRELKDLKGYG -COOH
YF-Q11-ZEp1	Ac-X-GG-YFQQKFQFQFEQQ-NH2 X-$(O2Oc)_2$- VNELPHGWKAWGKSYFVRAAKT-COOH

Standard amino acids are represented by conventional one letter code. C(Cy3): Cyanine3-conjugated cysteine; C(Cy5): Cyanine5-conjugated cysteine; K(N_3): azidolysine; Prg: propargylglycine; (O_2Oc) :8-amino-3,6-dioxaoctanoic acid; X—X: means triazole bridge bound sequences

*V5: V5 Epitope Tag Peptide is a tag peptide derived from a small epitope present on the P and V proteins of the paramyxovirus of simian virus 5
**LAC: sequence derived from the milk protein alpha-lactalbumin according to H. Hochwallner, U. Schulmeister, I. Swoboda, M. Focke-Tejkl, V. Civaj, N. Balic, et al., Visualization of clustered IgE epitopes on alpha-lactalbumin, J. Allergy Clin. Immunol. 125 (2010).

Table S2. Peptides characterization summary

Code	HPLC Rt (min)	MS calc.	MS found
YF-Q11	10.24	$[M+1]^+$=1838.6, $[M+2]^{2+}$= 919.8	
YF-Q11-Cy3	12.46	$[M+1]^+$=2555.1, $[M+2]^{2+}$= 1278.0, $[M+3]^{3+}$=852.4	$[M+2]^{2+}$= 1277.7, $[M+3]^{3+}$=852.6
V5-Cy3	10.82	$[M+1]^+$=2197.0, $[M+2]^{2+}$= 1099.0, $[M+3]^{3+}$=733.0	$[M+2]^{2+}$= 1098.7, $[M+3]^{3+}$=733.4
LAC-Cy5	10.04	$[M+1]^+$=3025.4, $[M+2]^{2+}$= 1513.2,	$[M+2]^{2+}$= 1513.0, $[M+3]^{3+}$=1009.3,

		$[M+3]^{3+}$=1009.1, $[M+4]^{4+}$=757.1	$[M+4]^{4+}$=756.9
YF-Q11-N_3	9.66	$[M+1]^{+}$=2131.9, $[M+2]^{2+}$= 1066.4, $[M+3]^{3+}$=711.3	$[M+1]^{+}$=2132.1, $[M+2]^{2+}$= 1066.2, $[M+3]^{3+}$=711.0
V5	10.11	$[M+1]^{+}$=1808.8, $[M+2]^{2+}$= 904.9, $[M+3]^{3+}$=603.6	$[M+1]^{+}$=1808.6, $[M+2]^{2+}$= 904.5, $[M+3]^{3+}$=603.8
LAC	9.36	$[M+1]^{+}$=2609.3, $[M+2]^{2+}$= 1305.1, $[M+3]^{3+}$=870.4	$[M+2]^{2+}$= 1305.3, $[M+3]^{3+}$=870.1
ZEp1	9.23	$[M+1]^{+}$=2930.9, $[M+2]^{2+}$= 1465.6, $[M+3]^{3+}$=977.4, $[M+4]^{4+}$=733.3	$[M+2]^{2+}$=1465.3, $[M+3]^{3+}$=977.1, $[M+4]^{4+}$=733.0
YF-Q11-V5	10.25	$[M+1]^{+}$=3939.8, $[M+2]^{2+}$= 1970.4, $[M+3]^{3+}$=1313.9, $[M+4]^{4+}$=985.7	$[M+2]^{2+}$= 1970.7, $[M+3]^{3+}$=1314.0, $[M+4]^{4+}$=985.9
YF-Q11-LAC	9.44	$[M+1]^{+}$=4740.3, $[M+2]^{2+}$= 2370.6, $[M+3]^{3+}$=1580.8, $[M+4]^{4+}$=1185.8, $[M+5]5^{+}$=948.8	$[M+3]^{3+}$=1581.0, $[M+4]^{4+}$=1185.7, $[M+5]^{5+}$=948.6
YF-Q11-ZEp1	9.37	$[M+1]^{+}$=5061.9, $[M+2]^{2+}$= 2531.6, $[M+3]^{3+}$=1687.9, $[M+4]^{4+}$=1266.2, $[M+5]^{5+}$=1013.1, $[M+6]^{6+}$=844.5	$[M+3]^{3+}$=1687.7, $[M+4]^{4+}$=1266.0, $[M+5]^{5+}$=1012.9, $[M+6]^{6+}$=844.6

Table S3. Demographic information about the panel of Zika positive samples.

Internal ID	***Sex***	***Geographic Region***	***AGE***
01	F	Dominican Republic	22
03	M	Dominican Republic	45
04	F	Dominican Republic	51
05	M	Dominican Republic	39
06	F	Dominican Republic	42
07	M	Dominican Republic	22
08	M	Dominican Republic	31
09	M	Dominican Republic	36
10	F	Dominican Republic	27
11	M	Dominican Republic	55
12	M	Dominican Republic	53

Table S4. Demographic information about the panel of Healthy controls.

Internal ID	***Cathegory***	***Sex***	***Age***
H00042	Healthy donor	F	22
H00031	Healthy donor	M	45
H00280	Healthy donor	F	51
H00021	Healthy donor	M	40
H00211	Healthy donor	F	41
H00022	Healthy donor	M	22
H00068	Healthy donor	M	31
H00007	Healthy donor	M	36
H00071	Healthy donor	F	27
H00037	Healthy donor	M	55
H00020	Healthy donor	M	53
H00080	Healthy donor	F	29