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High sensitive and direct fluorescence detection of single viral DNA sequences by integration of double strand probes onto microgels particles

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Author Contributions

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Supplementary material

Supplementary methods

Reagents and Chemicals

Poly(ethylene glycol) dimethacrylate average Mn 550 (PEGDMA), Acrylic acid (AAc), Potassium persulfate (KPS), Fluoresceine O-methacrylate, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and Polyvinyl alcohol 40-88 (PVA), Dimethyl Sulfoxide (DMSO), Sodium Hydroxide and 2-(*N*-morpholino)ethanesulfonic acid (MES) were all purchased from Sigma-Aldrich and used as received. The dye Methacryloxyethyl thiocarbonyl rhodamine B was obtained from Polyscience Inc. Tris buffer 1M, pH 8 was supplied by Applichem GmbH. DNA oligonucleotides were purchased from Diatech Pharmacogenetics srl with HPLC purification (Table S1). Bovine serum albumin was supplied by Lonza.

Double strand probe set up and validation

Optimization Quencher/fluorophore ratio. 20 pmol of HIV tail-Cy5 were mixed at a 0.5-to-10 ratio of HIV quencher in Tris HCl pH 8 buffer in a final volume of 200 µL. Each sample was loaded onto a 96-well microplate and the fluorescence emission intensity was measured in 2300 EnSpire multilabel reader (Perkin-Elmer, Waltham, MA) by setting the λex_{Cy5}=633 and λem_{Cy5}=654. The residual Cy5 emission intensities upon the quenching event were normalized *vs.* the DNA tail Cy5 emission for each ratio point. The experimental uncertainty represents the standard error of the mean of three replicate assays.

Quenching and displacement kinetic of HIV DNA probe in homogeneous assay

Quenching kinetic of HIV DNA target in homogeneous assay. 20 pmol of tail-Cy5 DNA were mixed to 20 pmol of quencher DNA (molar ratio 1/1) in Tris HCl, pH 8 buffer in a final volume of 200 µL. The fluorescence quenching was monitored at least every 60 min until no variation in fluorescence recovery was recorded. The residual Cy5 emission intensities upon the quenching event were normalized *vs*. the DNA tail Cy5 emission for each ratio point. The experimental uncertainty represents the standard error of the mean of three replicates.
Displacement kinetic of HIV DNA target in homogeneous assay. 20 pmol of tail-Cy5 DNA were mixed to 20 pmol of quencher DNA in Tris HCl, pH 8 buffer. For each assay 20 pmol of HIV-DNA target were added to such solutions in a final volume of 200 µL and the fluorescence recovery was monitored at least every 60 min until no variation in fluorescence recovery was recorded. Quenched samples were used as a reference in order to evaluate the displacement efficiency. The Cy5 emission intensities upon the displacement event were normalized *vs*. the DNA tail Cy5 emission for each time point. The experimental uncertainty represents the standard error of the mean of three replicates.

Microgel characterization

COOH content. Potentiometric titrations were performed using Compact Titrator G20 (Mettler Toledo AG, Analytical Schwerzenbach, CH). Samples were prepared by suspending 0.050g of microgel in 50 mL of 10^{-3}M KCl solution. Titrations were run in a thoroughly cleaned 100 mL beaker equipped with a pH electrode while NaOH (0.1 M, freshly prepared from Standard volumetric concentrates) was used as titrant. During the titration, pH was measured as the function of the volume of delivered standard NaOH solution. 50 µL of titrant was delivered into the microgel dispersions, followed by magnetic stirring until the pH value was stable and recorded. The total volume of standard NaOH solution delivered at equivalence point was used to calculate the carboxyl content. [1,2]. The carboxyl groups content of the microgel is in a range of 1.1 ± 0.2 µmol/mg particles. As for Hoare *et al* [1,2] from the electrophoretic mobility results, the total number of charges attributable to -COOH groups on the surface of each microgel (Table S1) can be approximated using hard sphere colloid equations. [1,2] The measured electrophoretic mobility is related to the zeta potential through the Henry equation (1).

$$\zeta = 3\mu\eta \, / \, 2\varepsilon_0\varepsilon_r f(\kappa R) \qquad \textbf{(1)}$$

Here, R is the microgel radius, η is the solution viscosity, k is the inverse Debye length, ε_0 is the permittivity of a vacuum, ε_r is the medium dielectric constant, and $f(\kappa R)$ is Henry's function for a 1:1 electrolyte solution.

Using the zeta potential as an approximation of the surface potential $\Psi 0$, the surface charge density (σ) can be estimated using the Grahame equation **(2)**

$$\sigma=\zeta\varepsilon_0\varepsilon_r(\kappa R+1) \qquad \textbf{(2)}$$

The total number of charges on the surface of each microgel particle (Q) can subsequently be estimated by multiplying by the surface area of the microgel and dividing by the elementary charge; the final relationship is shown as **(3)**

$$Q= 6\pi R\mu\eta(\kappa R+1)/ e(f(\kappa R)) \qquad (3)$$

Microgel mass determination. Single microgel mass was determined from the intrinsic viscosity of different particle preparation by using a Ubbelohde viscometer as already described by Romeo *et al* [3]. The single microgel mass is on average $(1.2 \pm 0.2) \times 10^{-10}$ mg.

Microscopy acquisitions. TEM observation was performed by Tecnai G2 FEI operating at an acceleration voltage of 200 kV. The specimen was prepared as follows. One drop of dilute microgel suspension was cast on a copper EM grid covered with a thin holey carbon film and dried at room temperature. SEM was performed on a FE-SEM Ultra Plus (Zeiss) microscope at 20 kV. For sample preparation, the dialyzed microgels solution was fixed on a microscope slide, air-dried and then sputtered with a 10 nm thin gold layer.

Probe name	Sequence	Length (nt)	ΔG (Kcal mol^{-1})[a]
	HIV probes [b]		
HIV tail-Cy5	5’ Cy5 ACT GCT GTT AAA C6 NH_2-3’	12	Tail hybridization 11.2
HIV quencher	5’ TTT AAC AGC AG BHQ TGA GTT GAT ACT ACT GGC CTA ATT CCA 3’	39	Target hybridization 50.9
HIV-target	5’ TGG AAT TAG GCC AGT AGT ATC AAC TCA ACT GCT GTT AAA 3’	39	
			Displacement 39.7
	HCV probes [c]		
HCV tail-Cy5	5’ Cy5 TTC CGG TGT ACT-C6 NH_2-3’	12	Tail hybridization 13.3
HCV quencher	5’-AGT ACA CCG GABHQ TTG CCA GGA CGA CCG GGT CCT TT-3’	35	Target hybridization 53.7
HCV-target	5’- AAA GGA CCC GGT CGT CCT GGC AAT TCC GGT GTA CT -3’	35	
			Displacement 40.4
	SARS probes [d]		
SARS tail-Cy5	5’ Cy5 GGC TCC AGT ATA -C6 NH_2-3’	12	Tail hybridization 11.9
SARS quencher	5’- TAT ACT GGA GCBHQ ATT GTC TAC CTG AAC ACT ACC GCG T -3’	37	Target hybridization 52.4
SARS-target	5’- ACG CGG TAG TGT TCA GGT AGA CAA TGG CTC CAG TAT A -3’	37	
			Displacement 40.5

Supplementary Tables

Table S1. Sequence, modifications and thermodynamic parameters of DNA probes used in this study

HIV100 probes [e]			
HIV 100-R[a]	5'TGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTAAATGGCAGTCTAGCAGAAG AAGAGGTAGTAATTAGATCTGTCAATTTCACGGACAATGCTAA-3'	99	
HIV100-M[a]	5'TACAAATGTCAGCACAGTACAATGTACACATGGAATTAGGCCAGTAGTATCAACTC AACTGCTGTTAAATGGCAGTCTAGCAGAAGAAGAGGTAGTAAT-3'	99	
HIV 100-L[a]	5'TAATAAGACGTTCAATGGAACAGGACCATGTACAAATGTCAGCACAGTACAATGTA CACATGGAATTAGGCCAGTAGTATCAACTCAACTGCTGTTAAA-3'	99	

[a] ΔG values are calculated using *Oligocalc* software (http://www.basic.northwestern.edu/biotools/oligocalc.html)
[b] HIV sequence source: HIV 1 (GenBank: AF033819.3 position 6520-6559 belonging to *env* gene coding for transmembrane envelope protein)
[c] HCV sequence source: HCV-1a (M67463.1 position 160-195
[d] SARS sequence source: Human coronavirus 229E, complete genome, (GenBank AF304460 position 16707-16743)

Table S2. Statistical analysis for LOD determination of ds displacement assay in homogeneous conditions or using microgels performed in presence of SARS, HCV and HIV DNA target

Assay	Target	Slope	Standard error (slope)	Intercept	Standard error (intercept)	LOD	R-Sq	F value	P>F	P<
ds displacement assay	SARS	$6.30*10^{9}$	$4.65*10^{6}$	415.17	0.28	133 pM	0.99	3880	$2.47*10^{-5}$	$2.0*10^{-5}$
	HCV	$1.77*10^{9}$	$9.07*10^{7}$	235.07	0.36	622 pM	0.95	52.36	$1.85*10^{-2}$	$1 *10^{-2}$
	HIV	$1.19*10^{9}$	$7.33*10^{6}$	232.36	0.37	928pM	0.98	297.51	$3.34*10^{-3}$	$3*10^{-2}$
Microgel based assay	SARS	$2.22*10^{16}$	$1.76*10^{15}$	538.1	10.1	1.40 fM	0.98	259	$5.11*10^{-4}$	$5*10^{-2}$
	HCV	$2.00*10^{16}$	$3.48*10^{15}$	550.5	24. 5	3.7 fM	0.99	636.20	$1.36*10^{-4}$	$3*10^{-2}$
	HIV	$3.21*10^{16}$	$3.44*10^{15}$	624	15.3	1.41 fM	0.95	74	$3*10^{-2}$	$1 *10^{-2}$

Table S3. Estimation of COOH groups on microgel surface at pH 5 and pH 9.

El. Mobility pH 5 (x10-8 m2/Vs)	El. Mobility pH 9 (x10-8 m2/Vs)	Surface charge pH 5 (n°COOH/microgel)	Surface charge pH 9 (n° COOH/microgel)
-0.85±0.006	-1.2±0.03	634±148	1130±99

Supplementary figures

Double strand probe set up and validation

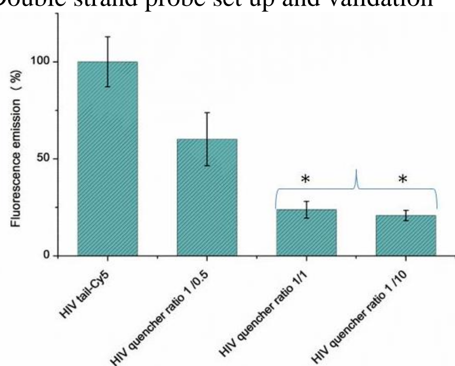


Fig. S1. Double strand probe set up and validation: HIV tail-Cy5/ HIV quencher ratio optimization.

Quenching and displacement kinetic of HIV DNA probe in homogeneous assay

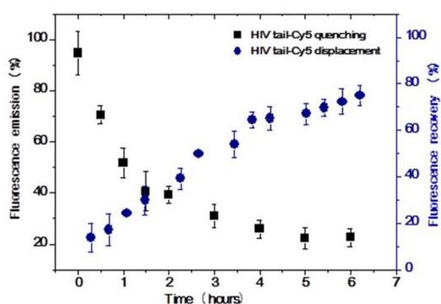


Fig. S2. Double strand probe set up and validation: kinetic study of incubation time for the quenching and displacement step of HIV probes. The Cy5 emission intensities were normalized *vs*. the HIV tail-Cy5 emission for each time point.

Electronic microscopy of microgels

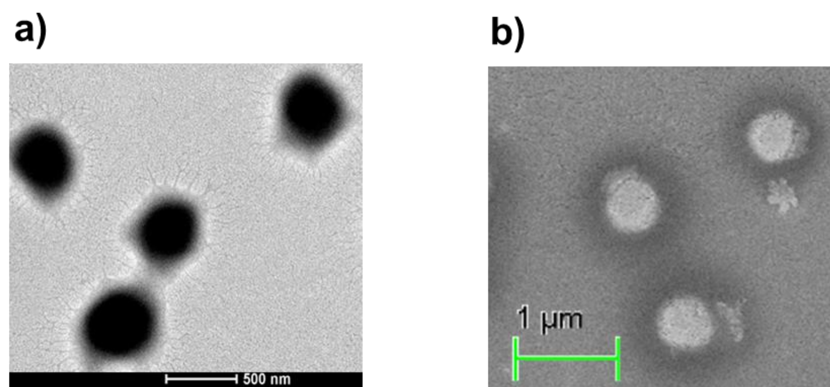
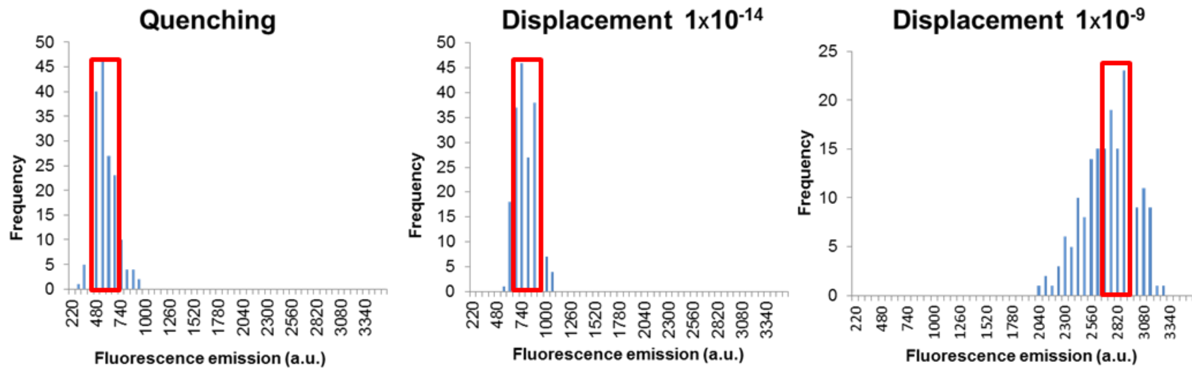


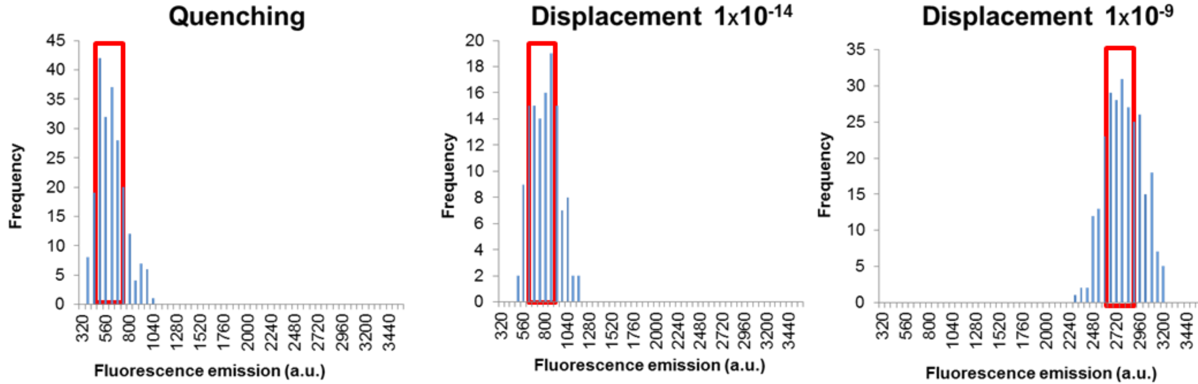
Fig. S3. Electronic Microscopy images of core double shell microgel. **(a)** TEM, **(b)** SEM.

Image analysis and elaboration

a) SARS



b) HCV



c) HIV

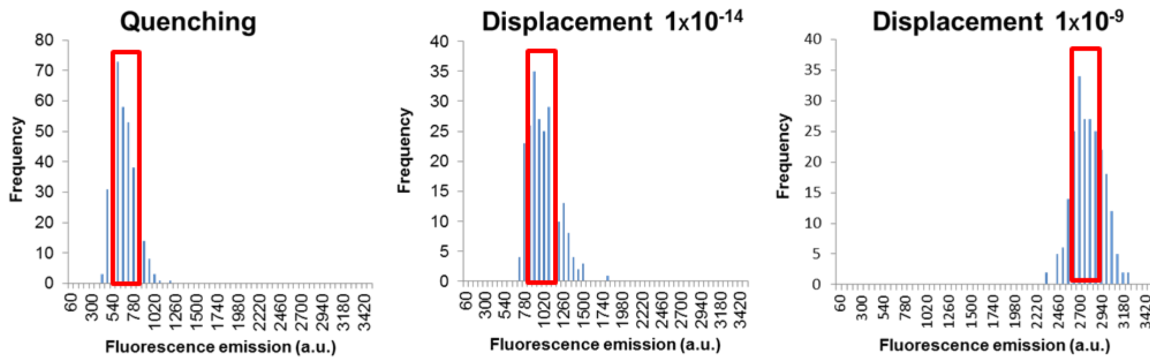


Figure S4 Fluorescence intensity distribution from a set of microgel particles upon contact and subsequent displacement at fixed target concentrations. Frequency occurrence is plotted as function of fluorescence emission at each conditions i) quenching, after tail hybridization with quenching sequences; ii) fluorescence recovery after displacement due to the target contact at concentration of $1x10^{-14}$ and $1x10^{-9}$ M in the case of a) SARS, b) HCV and c) HIV sequences.

References

[1] T. Hoare, and R. Pelton, **2004** *Langmuir.*, 20, 2123-2133.
[2] T. Hoare, and R. Pelton, **2008** *Curr Opin Colloid Interface Sci.*, 13, 413-428.
[3] G. Romeo, L. Imperiali, J. W. Kim, A. Fernandez-Nieves, and D. A. Weitz, **2012** *J Chem Phys*., 136, 124905.