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# Assembly and Characterizations of Bifunctional Fluorescent and Magnetic Microneedles Displaying Length Tunability over One Decade

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## Abstract

1  
2 We report on the fabrication of bifunctional magnetic and fluorescent microneedles  
3 ( $\mu$ NDS) made of a ternary mixture of magnetic nanoparticles (NPs), quantum dots  
4 (QDs) and polyelectrolyte. The assembly relies on the electrostatic complexation of  
5 negatively charged NPs with positively charged polymer strands and is controlled by  
6 the charge ratio between the nanoparticulate building blocks and the polymer mor-  
7 tar. We show that the resulting  $\mu$ NDS retain the fluorescent and superparamagnetic

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8 properties of their NP constituents. Moreover, using a combination of core and sur-  
9 face characterisations and a state-of-the-art image analysis algorithm, we thoroughly  
10 investigate the dependence of the brightness and length on the ternary composition.  
11 In particular, based on statistics on hundreds of  $\mu$ NDs with a range of compositions,  
12 we show that the length can be robustly tuned over one decade to match the relevant  
13 length scales of various applications.

14 **Keywords:** microneedles, magnetic nanoparticles, quantum dots, nanoparticle assembly,  
15 confocal fluorescence microscopy

## 16 1 Introduction

17 1D-magnetic microstructures made of single magnetic microparticles or assemblies of mag-  
18 netic nanoparticles can find applications in a wide variety of fields that includes composite  
19 materials, microfluidics and micro-mechanics. Micro-rods of non-magnetic material coated  
20 with iron oxide nanoparticles have been used to mechanically reinforce polymer matrices  
21 through applying small magnetic fields [1]. Micro-machined pieces of magnetic materials or  
22 chains of magnetic colloids have been applied as micro-stir bars to increase the efficiency of  
23 mixing in microfluidic channels and micro-droplets [2–6]. Mixing at the microscale can also  
24 be enhanced using micropillars or artificial cilia consisting of magnetic micro or nanoparticle  
25 assemblies that can be actuated through application of a rotating or a swinging magnetic  
26 field [7]. These magnetic cilia can form the basis of flow or vibration sensing when coupled to  
27 a giant magnetoresistive sensing element that monitors their deflection [8,9]. Alternatively,  
28 the bending of these cilia under a static external field can serve as a mean to apply con-  
29 trolled stresses to soft materials deposited on the substrate such as tissues, films or cells in  
30 order to study their mechanical responses [10–12]. In the same line, free standing magnetic  
31 micro-rods can be used as active micro-rheometers to probe the viscoelastic properties of  
32 complex fluids, including living cells, with microscale resolution [13,14].

33 Many of these applications would benefit from the addition of a fluorescent functionality  
34 to the magnetic one. Incorporating fluorophores into the 1D-microstructures enables easier  
35 imaging in scattering media. It could allow for optical bar-coding of the magnetic micro-

36 actuators. In cell mechanics studies, it could give insights into the environment of the  
37 magnetic probe through colocalisation experiments involving organelle-specific fluorophores.  
38 Moreover, most of these applications simultaneously require the length of the 1D-magnetic  
39 microstructures to be adjusted to the problem at play. Micro-stirrers need to be attuned  
40 to the dimensions of the fluid compartment and to its viscosity. The range of forces to  
41 which magnetic microposts are sensitive depends on their length and thickness. To be  
42 able to resolve inhomogeneities in viscoelastic properties at the microscale, magnetic micro-  
43 rheometers need to be of dimensions comparable to the typical length scale of the structural  
44 inhomogeneities [15, 16].

45 Bifunctional magnetic and fluorescent nano or microspheres have been reported in the  
46 past. They were developed mostly towards applications in bimodal magnetic resonance and  
47 fluorescence imaging, drug delivery or bioassays.

48 These materials have been synthesised according to various strategies: core-shell parti-  
49 cles, where a core of magnetic material (*e.g.* iron oxide nanoparticles) is encased in a shell  
50 of fluorescent one (*e.g.* dye-labelled silica or quantum dots) or the other way round [17–19];  
51 raspberry-like particles, where a large particles of one material are decorated on their sur-  
52 face with smaller particles of the other material [20, 21]; colloidosomes self-assembled from  
53 fluorescent and magnetic colloids [22]; beads in which particles of both materials have been  
54 coprecipitated or co-embedded in a silica or polymer matrix [23–25]; and particles made of  
55 oxides of rare earth ions [26]. Most of these strategies lead to bifunctional spheres in the 0.1  
56 to 1  $\mu\text{m}$  range whose size cannot be easily tuned. In general, they are not amenable to syn-  
57 thesize 1D structures. Moreover, in many cases the synthetic cost is high, either because of  
58 the minute scale of production or to the time or number of steps required. We report here on  
59 the fabrication of bifunctional magnetic and fluorescent 1D-structures of micrometric length  
60 assembled by controlled aggregation of a ternary mixture of polyelectrolyte, superparamag-  
61 netic nanoparticles and quantum dots. The building blocks are simple and easily sourced.  
62 Assembly proceeds in water, in less than half an hour, to give rise to wire or needle-like  
63 aggregates of millions of nanoparticles that are responsive under an external magnetic field  
64 and can be imaged by conventional epifluorescence microscopy. Interestingly, by playing on  
65 the ternary composition, their length can be tuned in the 5-50  $\mu\text{m}$  range to match the needs



66 of many microfluidic or biophysical applications. As an illustration of the potential of the  
67 microneedles ( $\mu$ NDs) for cell mechanics studies, we show that they are readily internalised  
68 by HeLa cells and that their fluorescence can be used to assess their localisation with regards  
69 to cellular compartments.

## 70 **2 Results and discussion**

### 71 **2.1 General strategy for assembling fluorescent magnetic micronee-** 72 **dles**

#### 73 **2.1.1 Assembly of 1st generation $\mu$ NDs**

74 The bifunctional magnetic  $\mu$ NDs derive from a first generation of magnetic-only 1D-microstructures  
75 reported previously. This first generation of magnetic  $\mu$ NDs was obtained by electrostatic  
76 complexation of negatively charged magnetic nanoparticles (mNPs) with polycationic poly-  
77 mers in a kinetically controlled fashion [27–29]. The magnetic building blocks consisted of  
78 6-8 nm superparamagnetic  $\text{Fe}_2\text{O}_3$  maghemite NPs coated in a 3 nm-thick layer of sodium  
79 polyacrylate that imparted them a persistent colloidal stability, even at high ionic strength  
80 ( $\geq 1\text{M}$ ). Various polyelectrolytes bearing positive charges through ammoniums (either qua-  
81 ternary or proton-exchanging) were investigated and all lead to successful assembly [30].

82 Due to the strong driving force for electrostatic assembly, direct mixing of the NP build-  
83 ing blocks and polyelectrolyte glue resulted in macroscopic precipitation of large aggregates.  
84 The assembly protocol was therefore designed to slow down the interaction. To this end,  
85 the constituents were mixed in the presence of a screening electrolyte at high ionic strength  
86 (typically in the M range), at which stage they remained individually dispersed. Assembly  
87 was then driven by slow dialysis of the screening electrolyte to give rise to spherical clus-  
88 ters. When performing the assembly under a static magnetic field (typically of 0.3 T), 1D  
89 structures grew and lead to microneedles between 150 and 400 nm in width and 1 to 500  $\mu\text{m}$   
90 in length that displayed superparamagnetism inherited from their iron oxide constituents.  
91 Starting binary compositions were referenced using the charge ratio  $Z$ , defined as the number  
92 of negative charges brought by the mNPs divided by the number of positive charges brought

93 by the polymer (1).

$$Z = \frac{C_{mNP}^{\ominus} V_{mNP}}{C_{pol}^{\oplus} V_{pol}} \quad (1)$$

### 94 **2.1.2 Choice of constituents for fluorescent magnetic $\mu$ NDs**

95 One possible strategy to incorporate a fluorescent functionality into the magnetic  $\mu$ NDs  
96 described above consists in substituting fluorescent building blocks for a fraction of the  
97 magnetic ones. Fluorescent NPs then need to present the same chemical reactivity as mNPs  
98 with regards to the assembly mechanism. This implies that they must bear negative charges  
99 on their surface and, critically, display colloidal stability even at high ionic strength. With  
100 the aim of tracking the  $\mu$ NDs using fluorescence (video) microscopy, the  $\mu$ NDs should ideally  
101 be as bright as possible. Indeed, strong brightness not only increases contrast but also enables  
102 the use of short exposure times and therefore give access to shorter timescales of dynamic  
103 tracking. Moreover, the  $\mu$ NDs should display homogeneous fluorescence, , namely should be  
104 homogeneously doped with fluorescent blocks, so that the fluorescence profile tightly mirrors  
105 the shape of the object. In a solid state solution, doping is facilitated by a similarity of  
106 size between the dopant and the host. Based on the same principle, the fluorescent building  
107 blocks and the magnetic ones should have diameters of the same order of magnitude.

108 Taking into account these criteria, we selected the AgInS<sub>2</sub>–ZnS (ZAIS) quantum dots  
109 (QDs) described by Regulacio *et al.* [31] as fluorescent building blocks (Fig. 1). These hy-  
110 drosoluble QDs were synthesised directly in water and were a few nm in diameter. Their  
111 emission was composition-dependent and could be tuned from 525 to 640 nm, with a max-  
112 imum reported quantum yield of 20%. Interestingly, these QDs were synthesized in the  
113 presence of polyacrylic acid, which acted as a capping agent. Having a diameter within the  
114 same range as the mNPs and the exact same coating, we expected these QDs to be an ideal  
115 dopant for the  $\mu$ NDs.

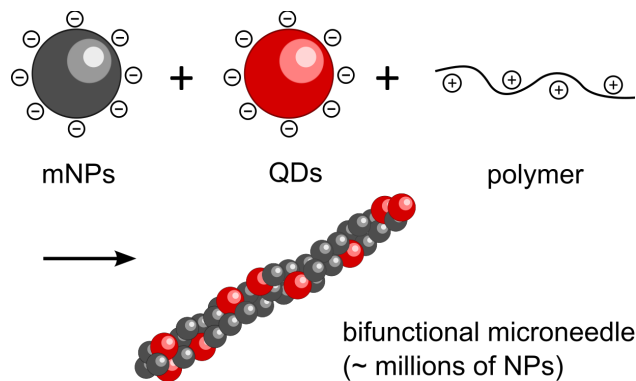


Figure 1: Synthetic scheme for assembly of bifunctional fluorescent magnetic  $\mu$ NDs

NPs	$D_c$ (nm)	$D_H$ (nm)	core	coating	charge density (mmol/g)
QDs	$5.8 \pm 1.2$	$\sim 13$	$\text{Ag}_{0.23}\text{In}_{0.58}\text{Zn}_{1.54}\text{S}_3$	PAA	$3.865 \pm 0.242$
mNPs	$9.4 \pm 1.7$	$\sim 31$	$\text{Fe}_2\text{O}_3$	PAA	$0.975 \pm 0.262$

Table 1: Summary of QD and mNP characteristics

### 2.1.3 Characterisations of magnetic and fluorescent building blocks for NW assembly

The mNPs used for  $\mu$ ND assembly were synthesised following the Massart method and coated in a post-synthetic fashion with PAA, as described earlier [32, 33]. The detailed characterizations of the exact batch used in this study are presented in ESI. Hereafter, we present the characterisations of the QDs we synthesized using the procedure from Regulacio *et al.* with few modifications, and compare the QD characteristics to those of the mNPs used for  $\mu$ ND assembly (Table 1) [31].

**Optical properties.** Iron oxide NPs present an intense and continuous absorption in the UV-vis range that vanishes close to 610 nm. We therefore aimed at synthesizing orange to red-emitting QDs to prevent fluorescence quenching through energy transfer to or re-absorption by the host matrix of the  $\mu$ ND. The synthesized QDs had a maximum emission peak centered at 600 nm and a moderate quantum yield of 7%, which was compensated by the emission sitting well outside the iron oxide absorption range (Figure 2). According to Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis, the

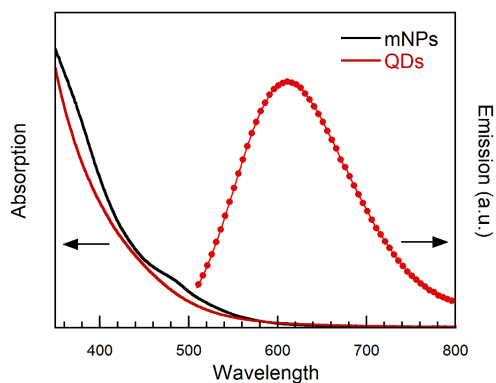


Figure 2: Absorption spectra of mNPs and QDs (plain) and emission spectrum of QDs under 490 nm irradiation (dots).

131 QDs had a non-stoichiometric  $\text{Ag}_{0.23}\text{In}_{0.58}\text{Zn}_{1.54}\text{S}_3$  formula, reflecting an excess chalcogen  
 132 compared to the metal ions and a defect in silver compared to indium, which is consistent  
 133 with orange emission (Table S1) [34].

134 **Size properties.** TEM images of QDs reveal a  $5.8 \pm 1.2$  nm core diameter based on  
 135 counting 175 NPs (Fig. S1). The hydrodynamic diameter as determined by DLS was 13 nm  
 136 in pure water at pH 8.5 (Fig. S2). The QDs and mNPs therefore had dimensions of the same  
 137 order of magnitude, both regarding their inorganic core and their size in solution. Impor-  
 138 tantly, the hydrodynamic diameter of QDs remained unchanged in the screening electrolyte  
 139 solution used for  $\mu\text{ND}$  assembly (1M  $\text{NH}_4\text{Cl}/\text{NH}_4\text{OH}$ ) over a period of days, evidencing  
 140 remarkable colloidal stability due to the PAA coating. Just as the mNPs, the QDs were  
 141 resilient towards the NW assembly conditions.

142 **Charge density and concentration.** As evidenced by gel electrophoresis on agarose  
 143 and consistently with PAA coating, the QDs were negatively charged (Fig. S9). They also  
 144 exhibited a higher number of surface carboxylates per core mass unit than the mNPs, a fact  
 145 that was confirmed by analysis of the composition of both batches of QDs and mNPs, by  
 146 ICP-OES for the inorganic core elemental concentrations and pH-titration for the surface  
 147 carboxylates (see ESI) [35]. Detailed compositions of the stock solutions are provided in  
 148 Tables S1 & S5.

## 149 2.2 Assembly of fluorescent magnetic microneedles

150 Assembly of fluorescent magnetic  $\mu$ NDs was performed following the same synthetic pro-  
 151 cedure as those developed previously for magnetic-only  $\mu$ NDs, but for the substitution of  
 152 QDs for a fraction  $w$  of mNPs (Eq. (2)). For a given assembly procedure, the starting  
 153 reagent stoichiometry is unambiguously defined by two synthetic parameters:  $w$  and the  
 154 previously defined  $Z$  ratio between negative charges brought by the nanoparticles and posi-  
 155 tive charges brought by the polymer, which now takes into account the contribution of QDs  
 156 (Eq. (3)). Alternatively, the ternary composition can be depicted using the molar fractions  
 157 of the charges respectively brought by QDs, mNPs and polymer, according to Equations  
 158 (4). A set of 15  $\{Z;w\}$  compositions with  $Z \in [0.62 - 7.01]$  and  $w \in [0 - 0.2]$  was inves-  
 159 tigated (Table S6). The individually dispersed mNPs are too small to be collected using a  
 160 table magnet but their aggregates have a much higher saturation magnetization. Successful  
 161 decantation under an applied magnetic field (typically of few hundredth of Tesla) therefore  
 162 evidences that assembly has taken place. According to this indicator, we observed that all  
 163 investigated compositions successfully led to  $\mu$ ND assembly.

$$w = \frac{V_{QD}}{V_{mNP} + V_{QD}} \quad (2)$$

$$Z = \frac{C_{QD}^{\ominus} V_{QD} + C_{mNP}^{\ominus} V_{mNP}}{C_{pol}^{\oplus} V_{pol}} \quad (3)$$

$$\begin{aligned} x_{QD} &= \frac{C_{QD}^{\ominus}}{C_{QD}^{\ominus} + C_{mNP}^{\ominus} + C_{pol}^{\oplus}} \\ x_{mNP} &= \frac{C_{mNP}^{\ominus}}{C_{QD}^{\ominus} + C_{mNP}^{\ominus} + C_{pol}^{\oplus}} \\ x_{pol} &= \frac{C_{pol}^{\oplus}}{C_{QD}^{\ominus} + C_{mNP}^{\ominus} + C_{pol}^{\oplus}} \end{aligned} \quad (4)$$

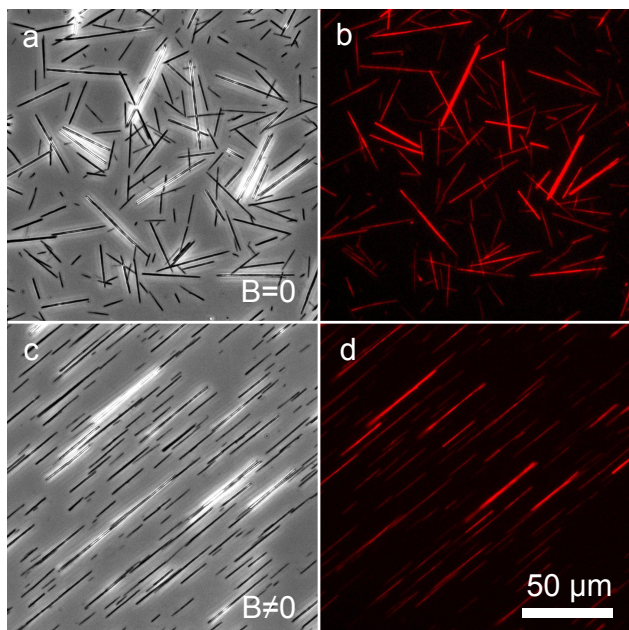


Figure 3: Phase contrast and epifluorescence microscopy images of  $\{Z = 3.62; w = 0.15\}$   $\mu$ NDs, without (a,b) and under (c,d) static magnetic field.

## 164 2.3 Properties of QD-doped magnetic $\mu$ NDs

### 165 2.3.1 Overview of properties: fluorescence and responsiveness under magnetic 166 actuation

167 Figure 3a depicts a phase 20 X contrast image of the aggregates obtained starting from the  
168  $\{Z = 3.62; w = 0.15\}$  composition. Straight  $\mu$ ND are obtained with lengths ranging from  
169 about 5 to 50  $\mu$ m and diameters of about 0.3-1.0  $\mu$ m (see ESI for TEM characterisation  
170 of width). Their morphology is identical to the magnetic-only  $\mu$ ND reported previously.

171 Figure 3b displays the same specimen area imaged by epifluorescence microscopy. The  $\mu$ ND  
172 emit in the red range under green excitation and appear homogeneously bright, evidencing  
173 homogeneous incorporation of the QDs in the host  $\mu$ ND matrix and retention of their optical  
174 properties. When depositing a soft permanent magnet on the microscope stage, the  $\mu$ ND  
175 respond to the external magnetic actuation by aligning along the field lines (Fig. 3c &  
176 3d). Upon suppressing the external magnetic field, the  $\mu$ ND relax towards randomized  
177 orientations, thereby evidencing superparamagnetism.

178 Interestingly, the morphology and in particular the length of the  $\mu$ NDs vary with the

179 starting composition (Fig. 4). While  $\{Z = 4.20; w = 0.20\}$ - $\mu$ NDs have a mean length of 13  
180  $\mu\text{m}$ ,  $\{Z = 1.40; w = 0.20\}$ - $\mu$ NDs that have the same QDs substitution ratio are on average  
181 3 times longer (39  $\mu\text{m}$ ). Both samples display straight persistent needle-like aggregates. In  
182 contrast, the  $\{Z = 1.21; w = 0.15\}$  sample contains large, soft, hair strand-like bundles of  
183  $\mu$ NDs whose length cannot be systematically determined because of inter- $\mu$ NDs aggregation,  
184 along with some individual  $\mu$ NDs. In addition, the longer the  $\mu$ NDs or bundles of  $\mu$ NDs, the  
185 brighter they are. As both the length of the  $\mu$ NDs and their brightness ultimately determine  
186 their potential application, a systematic investigation of these parameters as a function of  
187 ternary composition was conducted.

### 188 **2.3.2 Systematic investigation of length and brightness as a function of ternary** 189 **composition**

190 **Tools for image analysis.** The distribution of length was analysed from phase contrast  
191 microscopy images acquired with 10, 20 and 40X objectives. Lengths were extracted using a  
192 custom-written, dedicated image analysis Matlab code. The algorithm for  $\mu$ ND recognition  
193 was based on the linear Hough transform, which is a feature extraction technique devoted to  
194 the identification of lines [36]. The software is successful in recognizing single objects in an  
195 entangled bunch of  $\mu$ NDs but not when the  $\mu$ NDs are bundled (Fig. S11). It is biased against  
196 smaller  $\mu$ NDs due to their comparatively lower contrast. The software features a graphical  
197 user interface (GUI) for quick and handy correction of the most obvious errors, such as  $\mu$ NDs  
198 identified shorter than they are due to uneven contrast along their length or wires aligned  
199 tip-to-tip mistaken for one long object. Using this automated image analysis tool, 750  $\mu$ NDs  
200 on average were counted per sample comprising individual  $\mu$ NDs, with no less than 200  $\mu$ NDs  
201 counted for that having the highest mean length. All length distributions were log-normal  
202 with relative standard deviations ranging from 15 to 23% (Fig. 4 & S12). Fluorescence  
203 analysis was performed based on epifluorescence microscopy images acquired with a single  
204 20X objective. The brightness of each sample was taken as the mean background-corrected  
205 intensity of the identified bright pixels in the set of sample images. The dependence of  
206 brightness and mean length on ternary composition are depicted in Fig. 5 (see also Table  
207 S7 and Fig. S14)

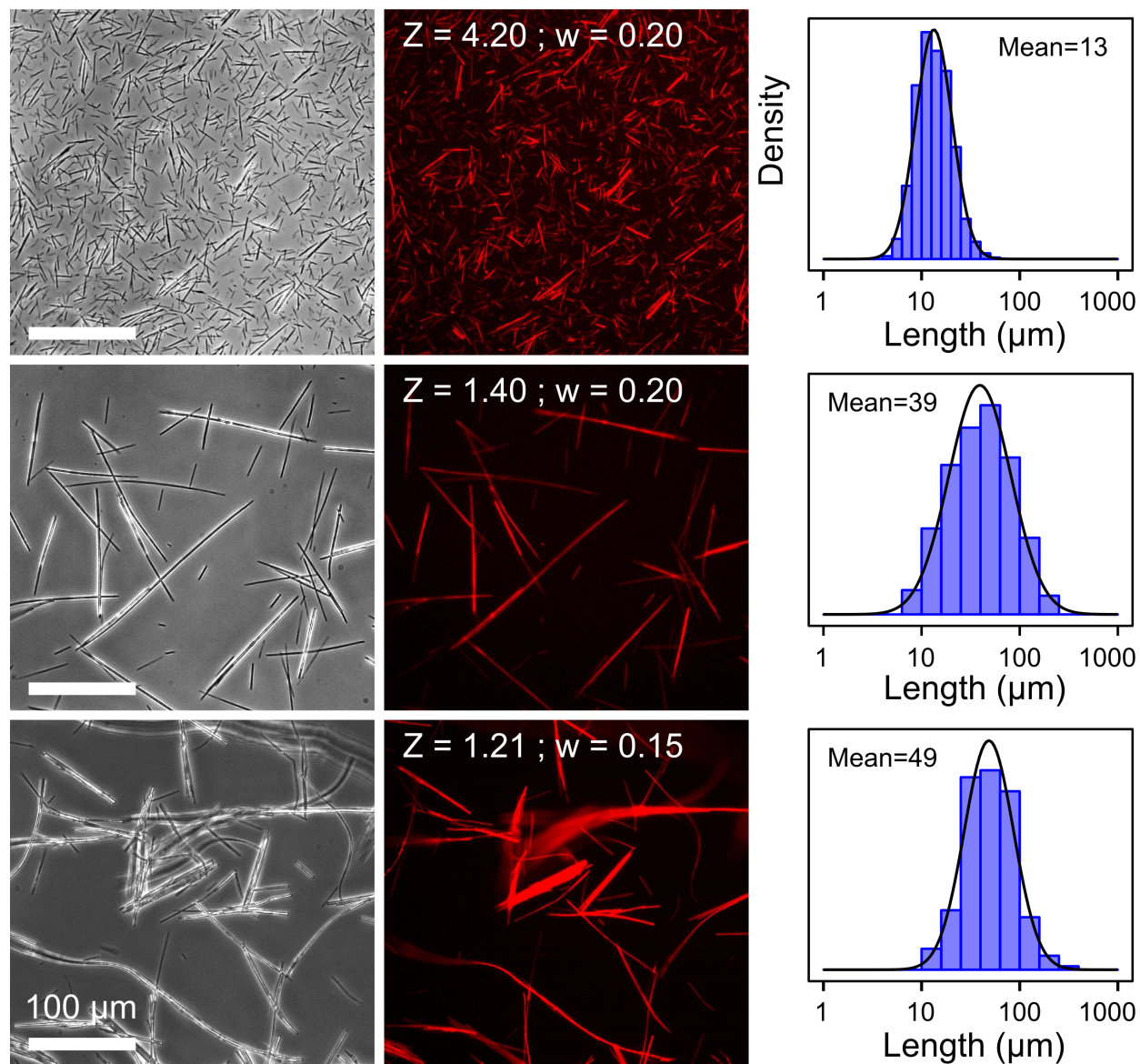


Figure 4: Phase contrast (*left*), epifluorescence (*middle*) microscopy images and associated length distributions (*right*) of bifunctional  $\mu$ NDs with various compositions.



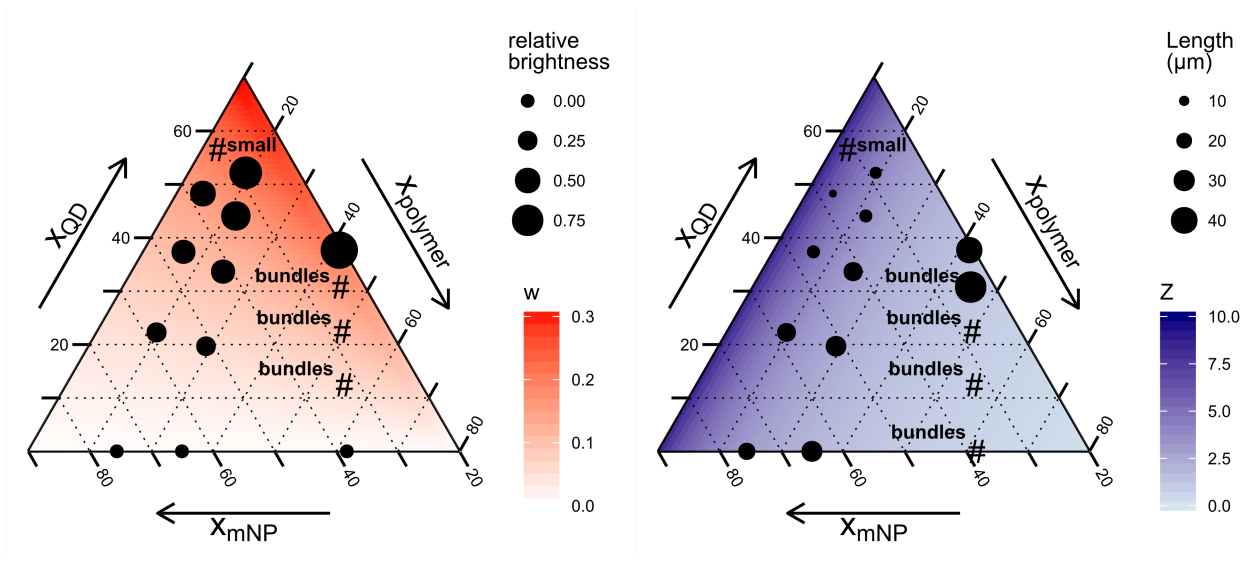


Figure 5: Relative fluorescence brightness (left) and mean length (right) as a function of ternary molar composition.

208 **Tuning of brightness.** For convenience, the QD-doping ratio  $w$  is mapped over the  
 209 range of ternary compositions in Figure 5 (left). As a general trend, starting compositions  
 210 richer in QDs display higher relative brightness. This observation evidences that the ac-  
 211 tual incorporation ratio parallels the starting doping ratio and supports the hypothesized  
 212 similarity in reactivity between PAA-coated QDs and PAA-coated mNPs.

213 **Tuning of length.** Out of 15 compositions, the mean length of 11 samples could be  
 214 extracted. Mapping the  $Z$  ratio over the ternary diagram reveals that the length decreases  
 215 with increasing  $Z$ , with the geometric mean length ranging from 5.7 to 48.5  $\mu\text{m}$  for  $Z = 6.03$   
 216 to 1.21 respectively. Three compositions lead to large bundles of aggregated  $\mu\text{NDs}$  whose  
 217 length could not be extracted; they corresponded to the weakest  $Z$  ratios across the whole  
 218 range of molar fractions ( $Z=0.62$ , 0.82 & 1.01 respectively). The last remaining sample  
 219 corresponded to the highest  $Z$  value (7.01). For this sample, the  $\mu\text{NDs}$  could not be resolved  
 220 using the 40X objective, although assembly had indeed taken place since the product could  
 221 be magnetically decanted with a table magnet, albeit admittedly very slowly.

222 The dependence of the mean length on the composition was rationalised earlier for  
 223 magnetic-only  $\mu\text{NDs}$  [28]. The assembly of  $\mu\text{NDs}$  was shown to occur in two steps that  
 224 are not separated in time (Fig. S10). In a first nucleation step, supra-colloidal clusters are

225 formed due to the electrostatic complexation between negatively charged mNPs and posi-  
 226 tively charged polymer strands once the ionic strength falls below a certain threshold. In  
 227 a second growth step, clusters align along the magnetic field lines, assemble and then fuse  
 228 due to magnetic dipolar attraction (Fig. S10). The surface charge of the clusters was found  
 229 to mirror the  $Z$  ratio of the starting mixture, with compositions featuring excess mNPs  
 230 ( $Z > 1$ ) leading to negatively charged clusters and those featuring an excess polycation  
 231 ( $Z < 1$ ) leading to positively-charged ones [30]. The kinetics of assembly therefore results  
 232 from the balance between dipolar magnetic attraction and electrostatic repulsion between  
 233 the supra-colloidal clusters. Accordingly,  $Z=1$ -magnetic-only  $\mu$ NDs, which stem from in-  
 234 termediate clusters having the weakest surface charge, were systematically found to be the  
 235 largest [30].

236 Here, we attempted at modelling the dependency of the length on compositional param-  
 237 eters in the case of the bifunctional  $\mu$ NDs. The dependence of the length on the  $Z$  ratio  
 238 in fluorescent magnetic  $\mu$ NDs appears fully consistent with the afore-mentioned mechanism.  
 239 There is strong anticorrelation at the 95% confidence level between length and  $Z$  ratio (see  
 240 ESI for details of the t-test). The shortest  $\mu$ NDs correspond to compositions having the  
 241 largest  $Z$  absolute values due to the increased electrostatic repulsion between clusters. As  
 242 the QD building blocks have a higher surface charge density than the mNPs, these shortest  
 243  $\mu$ NDs are also the richest in QDs. Since ZAIS QDs are diamagnetic, their incorporation  
 244 should lessen the saturation magnetisation of the clusters. For a given  $Z$  value, a higher QD  
 245 doping ratio should therefore lead to less intense magnetic dipolar attraction between clus-  
 246 ters. One might expect this phenomenon to slow down the assembly and lower the length  
 247 of the resulting  $\mu$ NDs. However, a t-test conducted on the data indicated no significant  
 248 correlation between the length and the doping ratio  $w$  (see ESI). Finally, the dependency  
 249 of the length on the compositional parameters could be simply described as Equation (5)  
 250 (Figure 6).

$$L \approx 65 \times \exp(-0.33Z) \quad (5)$$

251 where  $L$  is the geometric mean length and  $Z$  varies in the 1.2 - 6.0 range (Akaike infor-  
 252 mation criterion was applied to choose the best model, see ESI).

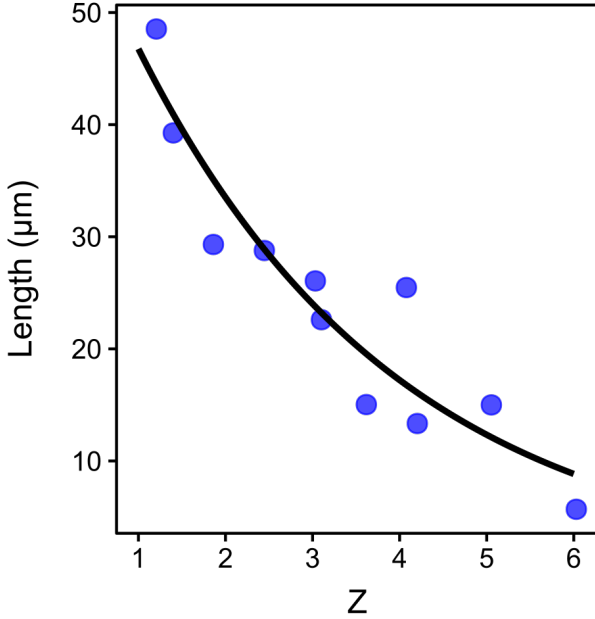


Figure 6: Geometric mean length of  $\mu$ NDs as a function of  $Z$  (blue dots) and best fit line according to Eq. (5) (black line).

253 Because of their length tunability in the 5 - 50  $\mu\text{m}$  range and the ease with which they can  
 254 be assembled, the bifunctional fluorescent magnetic  $\mu$ NDs could find applications in multiple  
 255 domains. Microfluidic channels have a typical width of 50 - 200  $\mu\text{m}$ . At the upper end of the  
 256 size window, 30-50  $\mu\text{m}$   $\mu$ NDs could be used as individual micro-stir bars as a cheaper alter-  
 257 native to micromachined devices that have been reported earlier [5]. Shorter  $\mu$ NDs could be  
 258 easily incorporated as multiple stir bars in arrays of microdroplets to efficiently churn up the  
 259 whole solution compartments [3, 4]. The magnetic actuation and fluorescent features could  
 260 be exploited in simultaneous magnetic and fluorescence-assisted cell sorting [37]. Alterna-  
 261 tively, the bifunctional  $\mu$ NDs could act as probes for active viscosimetry-based label-free  
 262 bioassays [38]. In both of these assaying applications, the synthetic control over the length  
 263 would enable fine tuning of probe to the application through optimisation of the saturation  
 264 magnetisation of the  $\mu$ NDs. Finally,  $\mu$ NDs could allow to probe the mechanical properties  
 265 of biological materials such as tissues, films, individual cells or subcellular compartments  
 266 using magnetic actuation-based techniques coupled to fluorescence imaging. Lately, the vis-  
 267 coelastic properties of cells and tissues have attracted much attention in the frame of medical

268 research. For instance, the mechanical stiffness of cancer cells has been shown to correlate  
269 with their metastatic potential, with softer more deformable cells having the highest migra-  
270 tory potential and invasiveness [39]. Moreover, cell state or fate can be controlled by the  
271 viscoelastic properties of its environment [40]. The phenomena at play are difficult to study  
272 by conventional macroscopic rheological methods since the samples typically come as tiny  
273 specimens and biological fluids are generally complex, *ie.* they behave differently at different  
274 length scales. In this context, the size tunability over the 5 - 50  $\mu\text{m}$  range is especially  
275 appealing since it would enable the study of a given material from the scale of assemblies of  
276 cells down to the subcellular level with a single technique suited to microscopic specimens.  
277 Such multiscale study would shine light on the respective contributions and interplay of the  
278 mechanics of cells, organites and surrounding matrix.

## 279 **2.4 Intracellular imaging of fluorescent magnetic microneedles**

280 Since many of these applications would rely on interaction of the bifunctional  $\mu\text{NDs}$  with  
281 cells and subsequent fluorescent imaging, we hereafter examine whether the present bifunc-  
282 tional fluorescent magnetic  $\mu\text{NDs}$  would be suitable to probe individual cells via confocal  
283 fluorescence microscopy. Magnetic-only  $\mu\text{NDs}$  were previously found to be internalised by  
284 mouse fibroblasts and human cancer cells, a phenomenon which was used to extract vis-  
285 coelastic properties of these cells through rotational magnetic spectroscopy [13]. Here, HeLa  
286 were incubated with QD-doped  $\mu\text{NDs}$ , fixed and stained with DAPI in order to fluorescently  
287 label their nucleus. Figure 7 displays bright field (*first row*) and fluorescence (*middle row*)  
288 confocal images acquired in two focal plans lying 6  $\mu\text{m}$  apart. The blue and red fluorescence  
289 channels correspond to DAPI and QD emissions, respectively. In the left panel, the nuclei  
290 are out of focus while they come into focus in the right panel. On bright field images, a few  
291  $\mu\text{ND}$  can be spotted, mostly in the right panel, but their localisation with regards to the  
292 cellular compartments is difficult to assess. On fluorescence images (*middle row*),  $\mu\text{NDs}$  can  
293 be more easily outlined as red dashes. On cell **C1** in left panel, a few  $\mu\text{NDs}$  appear on top of  
294 the out-of-focus nucleus. These  $\mu\text{NDs}$  disappear when the **C1**-nucleus comes into focus in the  
295 right panel, while many more  $\mu\text{NDs}$  with sharp contours appear around it. Reconstruction  
296 of the Z-profile of cell **C1** (*lower row*) conveniently shows that  $\mu\text{NDs}$  are internalised within

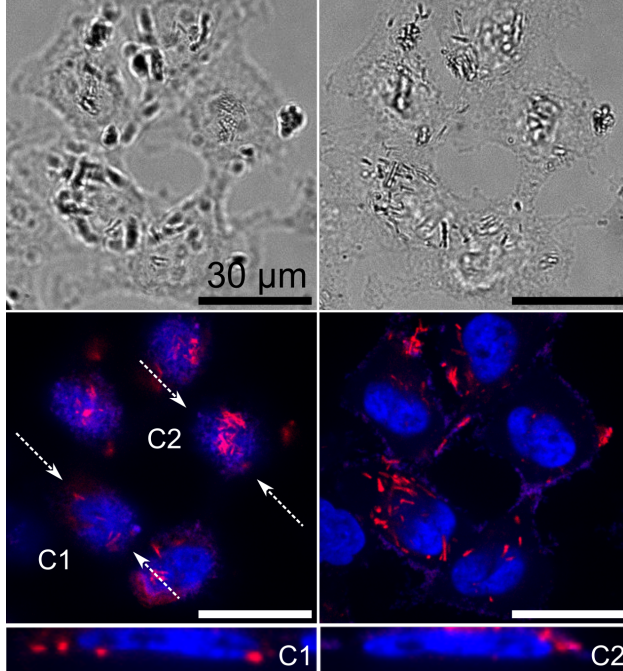


Figure 7: Bright field (upper row) and fluorescent (middle row) confocal images of HeLa cells incubated with bifunctional fluorescent magnetic  $\mu$ NDS (60X objective). Reconstructed Z-profiles of cells **C1** and **C2** in the planes perpendicular to the highlighted regions of interest (lower row).

297 the cytoplasm as the cell presents  $\mu$ NDS at nucleus height within its walls. On cell **C2** in left  
 298 panel, many bright  $\mu$ NDS appear on top of the out-of-focus nucleus, which disappear when  
 299 diving at nucleus height. These  $\mu$ NDS are most likely sitting on top of the cell or right on  
 300 top of the nucleus within the cell walls. The Z-profile of cell **C2** does not disambiguate the  
 301 situation as such but with fluorescent labeling of the cell membrane would. At any rate, the  
 302 fluorescence feature of the  $\mu$ NDS enables quick assertion of the localisation of the probes to  
 303 choose those to track to mechanically probe the rheological zones of interest.

### 304 **3 Summary**

305 In this work, bifunctional microneedles were readily assembled by electrostatic complexation  
 306 of a ternary mixture of superparamagnetic iron oxide nanoparticles, red-emitting zinc-silver-  
 307 indium sulfide quantum dots and a cationic polyelectrolyte. The microneedles encompass

308 the properties of both nanoparticulate components, namely superparamagnetism and flu-  
309 orescence in the visible range. They can be actuated using small external magnetic fields  
310 and imaged using epifluorescence or confocal fluorescence microscopy. Interestingly, we found  
311 that by playing on the ternary composition, more specifically on the ratio of negative charges  
312 brought by the nanoparticles to positive charges brought by the polyelectrolyte, the length  
313 of the microneedles could be tuned in the 5-50  $\mu\text{m}$  range. Because of their easy synthesis,  
314 their bifunctionality and their size tunability, these fluorescent and magnetic microneedles  
315 could find applications in multiple domains such as micromixing, bioassays or biomechanical  
316 studies and especially in the field of cell mechanics, since they were found to be readily  
317 internalised by model animal cells.

## 318 4 Methods

### 319 4.1 Synthesis and characterisation of nanoparticle building blocks

320 Maghemite mNPs were synthesized following the Massart procedure and surface-coated with  
321 sodium polyacrylate according to previously reported protocols [32, 33]. PAA-coated ZAIS  
322 QDs were synthesized in one step following the method reported by Regulacio *et al.* with  
323 minor modifications [31]. Core material concentrations were determined by ICP-OES for QDs  
324 and X-ray fluorescence for mNPs. Concentrations of surface carboxylates were determined by  
325 pH-titration with HCl. The quantum yield of QDs was determined by comparison to those of  
326 fluorescein and rhodamine 6G [41–43]. Gel electrophoresis was performed on 1%wt agarose  
327 gel in TBE 1x buffer. A full description of the synthetic and characterisation procedures is  
328 provided in Supporting Information.

### 329 4.2 Assembly of fluorescent magnetic microneedles

330 The following gives the synthetic procedure for assembly of  $\{Z = 4.20; w = 0.20\}$ - $\mu\text{NDs}$  as  
331 an example. First, solutions of building blocks in  $\text{NH}_4\text{Cl}$  1M pH 8 were prepared by mixing  
332 in a 1:1 ratio a  $\text{NH}_4\text{Cl}$  2M pH8 solution with the mNP (0.2% wt core, 2.02 mM carboxylate),  
333 the QD (0.4% wt core, 14.73 mM carboxylate) and polydiallyldimethylammonium chloride

334 (0.2% wt, 13.4 mM ammonium) stock solutions respectively. Next, 400  $\mu\text{l}$ , 100  $\mu\text{l}$  and 40.5  $\mu\text{l}$   
335 of mNP, QD and polymer dilute solutions were mixed in an eppendorf tube and shaken for  
336 10 s with the help of a vortex mixer. The ternary mixture was inserted in a 0.5 - 3 ml Slide-a-  
337 Lyzer dialysis cassette with a 10 kDa cutoff using a syringe. Air bubbles were removed. The  
338 cassette was nested into a custom made plexiglas sample holder sandwiched between two  $5 \times$   
339  $8 \text{ cm}^2$  0.3 T magnets. The sample holder was then immersed in a 2.5 L cylindrical flask filled  
340 with deionised water equipped with a stir bar and continuous monitoring of the conductivity  
341 was launched immediately. Dialysis was run for 2 hours after which the conductivity of  
342 the dialysis medium had reached a stable value. The content of the dialysis cassette had  
343 turned turbid, evidencing success of the assembly. The reaction media was carefully taken  
344 out using a syringe and inserted into a 2 ml glass vial.  $\mu\text{NDs}$  were sedimented using a soft  
345 table magnet and the supernatant was discarded. The  $\mu\text{NDs}$  were redispersed in 1 ml MilliQ  
346 water through gentle vortex mixing, sedimented again and the supernatant discarded. Two  
347 more washing steps using  $2 \times 1 \text{ mL}$  MilliQ water were performed. Finally, the  $\mu\text{NDs}$  were  
348 redispersed in 1 mL MilliQ water and stored in the dark at  $4^\circ\text{C}$ . The 14 other investigated  
349 compositions were prepared using the same protocol, with varying mNP, QD and polymer  
350 amounts, as given in Supporting Information.

### 351 **4.3 Imaging of $\mu\text{NDs}$ using phase contrast and epifluorescence mi-** 352 **croscopy**

353 A square well was built on a glass slide using double-sided tape. The NW suspension was  
354 homogenized and diluted 4 times with MilliQ water. 8  $\mu\text{l}$  of this diluted suspension were  
355 deposited in the well, which was then covered with a coverslip. The sample was imaged  
356 in phase contrast mode and in epifluorescence mode using excitation and emission filters  
357 suited for Rhodamine 6G, with 10X, 20X and 40X magnifications. Length and brightness  
358 distributions were extracted using custom-written Matlab codes described in Supporting  
359 Information.

## 4.4 Imaging of fluorescent magnetic $\mu$ NDs in interaction with P32 HeLa cells using confocal fluorescence microscopy

**Preparation of NW sample.** For incubation of cells, we selected  $\{Z=3.62, w=0.15\}$ - $\mu$ NDs. This sample had a mean length amongst the shortest in all investigated compositions (15  $\mu$ m). However, it was shown previously on a similar sized sample of magnetic-only  $\mu$ NDs that cells operate a size sorting of  $\mu$ NDs upon internalization, with an enrichment in  $\mu$ NDs having a length around 4  $\mu$ m [29]. We therefore shortened the  $\mu$ NDs by ultrasonication before cell incubation. To this end, 400  $\mu$ l were inserted in a 1 mm optical path quartz cuvette and sonicated for 2 X 1 min in an ultrasonic bath in horizontal position, with intermediate cooling for 5 min. The  $\mu$ NDs were sedimented in the cuvette using a table magnet and the supernatant was discarded. Next, they were washed 3 times with 500  $\mu$ l MilliQ water through sedimentation and discarding of the supernatant. Finally, they were dispersed in 500  $\mu$ l MilliQ water before autoclaving.

**Cell culture.** P32 HeLa cells were grown in T25-flasks as a monolayer in DMEM with high glucose (4.5 g/L) and stable glutamine (2 mM, PAA Laboratories GmbH, Austria). This medium was supplemented with 10%vt fetal bovine serum (FBS) and 1% penicillin/streptomycin (PAA Laboratories GmbH, Austria), referred to as cell culture medium. Exponentially growing cultures were maintained in a humidified atmosphere of 5% CO<sub>2</sub> and 95% air at 37 °C. In these conditions the plating efficiency was 70-90% and the cell duplication time was 12-14 h. Cell cultures were passaged twice weekly using trypsin-EDTA to detach the cells from their culture flasks and wells. For counting, the cells were pelleted by centrifugation at 1200 rpm for 5 min, supernatants were removed and cell pellets were resuspended in assay medium in Malassez counting chambers.

**Seeding of P32 HeLa cells onto microscope glas coverslips.** Sterilized microscope coverslips were nested in the wells of a 6-well plate, washed with 1 mL of PBS buffer and then with 1 mL of culture medium. Cells were detached from their culture flask and transferred to the wells; the cell concentration was 75 000 cells/ml and each well contained 2 mL of cell suspension. The cells were incubated for 24 hours to promote cell adhesion onto the coverslips.



389 **Incubation of P32 HeLa cells with  $\mu$ NDs.** The cell medium was exchanged against  
390 2 mL of PBS buffer and exchanged again for 1 mL of culture medium. Next, 15, 30 or 40  $\mu$ L  
391 of shortened  $\mu$ NDs were mixed with 1 mL of culture medium and the resulting suspension  
392 was added dropwise in each well. The cells were incubated with the  $\mu$ NDs for 20 hours after  
393 which they were washed by 2 rounds of medium exchange against PBS buffer.

394 **Cell fixation.** Next, the cells were fixed through incubation in 4% PFA solution (J61899,  
395 Alfa Aesar) in PBS for 20 min at room temperature. After copious rinsing of PFA using  
396 PBS, the nuclei were fluorescently stained with DAPI dilactate dye ( $\geq 98\%$ , Aldrich) by  
397 incubation for 20 min at room temperature in a 2.2  $\mu$ M dye solution in PBS. The medium  
398 was exchanged for PBS three times to remove excess dye. Finally, microscope slides were  
399 equipped with Gene Frame stickers. The coverslips were carefully taken out of the wells  
400 and mounted onto the Gene Frames after filling the wells with PBS buffer. Samples were  
401 examined with an inverted wide-field microscope (Olympus IX81) equipped with spinning  
402 disc module (Yokogawa CSU-X1), with an oil immersion objective (60X, NA 1.42) and an  
403 EMCCD camera (Andor iXon 897). Z-stacks of wide-field fluorescent images were acquired  
404 using a piezo at 0.5  $\mu$ m increments. For the DAPI channel, 405 nm laser-line excitation and  
405 a 465 nm-centered emission filter were used. For the NW channel, the excitation line was 488  
406 nm and the emission filter was centered at 607 nm.

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