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## Hydrogel embedded quantum dot-transcription factor sensor for quantitative progesterone detection

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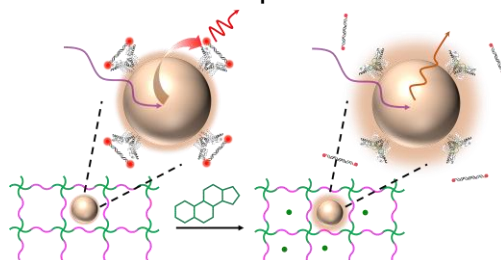
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**Keywords:** biosensing, quantum dots, transcription factor, hydrogel, Förster resonance energy transfer

**Abstract:** Immobilization of biosensors in or on a functional material is critical for subsequent device development and translation to wearable technology. Here we present the development and assessment of an immobilized quantum dot - transcription factor - nucleic acid complex for progesterone detection as a first step toward such device integration. The sensor is composed of a polyhistidine-tagged transcription factor linked to a quantum dot and a fluorophore-modified cognate DNA, and embedded within a hydrogel as an immobilization matrix. The hydrogel is optically transparent, soft, and flexible as well as traps the quantum dot - transcription factor DNA assembly but allows free passage of the analyte, progesterone. Upon progesterone exposure, DNA dissociates from the quantum dot - transcription factor DNA assembly resulting in an attenuated ratiometric fluorescent output via Förster resonance energy transfer. The sensor performs in a dose-dependent manner with a limit of detection of 55 nM. Repeat analyte measurements are also similarly successful. Our approach combines a systematically characterized hydrogel as an immobilization matrix and a transcription factor – DNA binding as a recognition/ transduction element, offering a promising framework for future biosensor devices based upon allosteric transcription factor.



## 1. INTRODUCTION

Digital health is positively transforming the life sciences from medical devices to diagnostics. Wearable sensor technologies will further drive the development of digital health and will provide a wealth of opportunities for detection and monitoring across the clinical landscape from cancer and trauma to addiction.<sup>1</sup> Yet, the full potential of digital health, and the subsequent benefit to patients, is not fully realized today because of the: 1) limited number antibodies, enzymes, and aptamers – *i.e.*, biorecognition elements – available for medical relevant biomarkers; and 2) need for additional strategies to integrate biorecognition elements within sensors. With regard to sensor discovery, a recent genomics-to-sensor approach taps directly into the repertoire of natural bacterial regulatory circuits, and identifies and harvests allosteric transcription factors (TF) for sensing. This new approach potentially addresses the problem of limited biosensors, and expands the number and types of existing biorecognition elements.<sup>2-4</sup> Results from these metagenomic sequence mining approaches are yielding new TFs as biorecognition elements for: 1) *in vivo* whole-cell sensors– *i.e.*, the bacteria itself is the biosensor;<sup>5-7</sup> and, 2) *in vitro* sensors– *i.e.*, the isolated bacterial TF is the sensing element in a biosensor.<sup>8</sup> <sup>9</sup> In particular, our group has designed quantum dot-based Förster resonance energy transfer (FRET) nanosensors for progesterone, where progesterone binding alters TF - operator DNA affinity affording a change in the proximity of the FRET donor (QD) and acceptor (fluorophore labeled DNA) with subsequent fluorescent signal detection.<sup>8</sup>

Progesterone, a steroid hormone of significant clinical relevance, is the primary biomarker of reproductive status and its monitoring in humans facilitates fertility planning and pregnancy.<sup>10</sup> Additionally, in cases of pregnancy of unknown location (PUL), progesterone is a validated biomarker of early gestation. Monitoring progesterone levels reduces the clinical burden of PUL cases and improves outcomes.<sup>11-12</sup> Measuring progesterone is also important in the livestock industry. For example, tracking the ovulation cycles of dairy cows and detecting pregnancy for fertility management leads to considerable cost saving for dairy farms.<sup>13</sup> Today, steroid hormones are quantified using HPLC or antibody-based immunoassays, and these analytical methods are ill-suited for real-time monitoring, repeat measurements, multiple sensing, or decentralized testing.<sup>14-17</sup>

The second challenge is integration of the discovered biorecognition element(s) and transduction mechanism into a device, a necessary step towards eventual use. Previously, QDs have been entrapped in hydrogels for phenol and dopamine chemo-/bio-sensing purposes.<sup>18-20</sup> Moreover, immobilization of the biorecognition elements or sensors enables working in flow conditions required for integration in lateral flow<sup>21-22</sup> or microfluidic devices.<sup>23-24</sup> Herein, we describe a QD-based FRET sensor for progesterone immobilized within a robust covalently crosslinked hydrogel matrix. Specifically, we adapted our previous solution phase QD-based FRET sensor,<sup>8</sup> composed of a polyhistidine-tagged transcription factor (SRTF1) self-assembled on the QD surface, and a Cy5 fluorophore-labeled cognate DNA sequence. Binding of the TF to its cognate DNA site in the absence of progesterone enables FRET between the QD and the Cy5; the presence of progesterone induces unbinding of the TF from the DNA with subsequent loss of FRET, yielding a progesterone concentration-dependent change in the fluorescence signal.<sup>8</sup> To our knowledge, this is the first report describing the integration of a transcription factor-based FRET sensor into a hydrogel and its subsequent performance, a critical step

towards device prototyping. Specifically, we report the: 1) preparation and characterization of the PEG hydrogel immobilization matrix; 2) sensor assembly; 3) diffusion studies of sensor constituents in the hydrogel; 4) sensor performance; and, 5) repeated progesterone measurements.

## 2. MATERIALS AND METHODS

Sterol responsive allosteric transcription factor (SRTF1-his<sub>6</sub>) and CdSe/CdS/ZnS QD were prepared according to a previously reported procedure.<sup>8, 25</sup> The QDs were transferred into water using a ligand-exchange procedure. The QD are stable in water of aqueous buffer, with a quantum yield of  $27 \pm 3\%$ .<sup>25</sup> Cy5-labeled HPLC-purified oligonucleotides were purchased from Integrated DNA Technologies, Inc. (Coralville, IA). Specifically, the polyhistidine-tagged transcription factor was self-assembled onto the QD surface at average ratios of TFs to QD (QD/TF=1/4) through chelation-based binding. Oligonucleotides labeled with Cy5-DNA containing SRTF1-his<sub>6</sub> binding sites were combined with QD-TFs at specified ratios of DNA to QD (QD/DNA=1/18).<sup>8, 25</sup> The QD emission spectrum overlaps with the Cy5-DNA excitation spectrum to enable FRET (Figure S9). The TF binding to oligonucleotides depends on hydrogen bonding and van der Waals interactions between TF DNA-binding domains and specific bases on oligonucleotides and this interaction is disrupted upon binding of an analyte – e.g., progesterone – resulting in separation of TF from DNA.<sup>26</sup> Metrics of sensor performance include EC<sub>50</sub> and limit of detection (LOD) values. EC<sub>50</sub> is the concentration of analyte that gives half-maximal response. The LOD is defined as the progesterone concentration yielding a signal greater than 3 times the pool standard deviations above background.

We synthesized the poly(ethylene glycol)-based hydrogels following published procedures.<sup>27-29</sup> Briefly, we mixed the 4-arm-PEG-NH<sub>2</sub> in 1X borate buffer at pH 8.6 with NHS-PEG-NHS, which contains the *N*-Hydroxysuccinimide functionalities, in phosphate buffered saline (PBS 1 x) at pH 7.4 to form the amide-bond linked network.

The sensor was dialyzed against progesterone (PRG) solution overnight (Figure 1A and B). With the presence of progesterone, the transcription factor unbinds its cognate DNA site. (Figure 1C and D). The sensor was then dialyzed against HEPES overnight to remove progesterone for another measurement. This cycle was repeated. Fluorescence measurements were recorded between cycles of progesterone exposure and removal. Fluorescence spectra were measured on a Nanolog spectrofluorometer (HORIBA, Ltd., NJ), equipped with a plate reader. The fluorescence intensity was monitored from 550 nm to 750 nm ( $\lambda_{\text{exc}} = 400 \text{ nm}$ ) with a 450 nm long-pass filter before the emission detector (Figure 1E). The intensity was recorded in relative fluorescence units, with baseline correction according to manufacturer's instruction.

All remaining experimental details, including characterization and assays, and statistical analysis are described in Supporting Information.

## 3. RESULTS AND DISCUSSION

Solution-phase sensors have merit, however integration of the design for analyte detection into immobilization matrix expands the potential application space (e.g., hydrogel electronics,<sup>30</sup> contact lens glucose sensor,<sup>31-32</sup> and hydrogel-based microsensors).<sup>33</sup> Additionally, successful incorporation of such sensors into lateral flow or microfluidic devices allows facile sample processing and precise flow control as well as integration with electronic devices for miniaturization.<sup>34</sup> As an initial step towards such devices, we recognize that our QD-based FRET sensors based on quantum dot-bound transcription factors and fluorophore-labeled nucleic acid duplexes are amenable to immobilization given the precedent for immobilized QDs in hydrogels for fluorescent probing and biocatalysis.<sup>18-19</sup> In the forthcoming text, we first describe the synthesis and characterization of the hydrogel, followed by the progesterone sensor construction and performance evaluation. Finally, we validated the performance of the sensor, by performing repeated measurements.

**3.1. Hydrogel Synthesis and Characterization.** We synthesized the poly(ethylene glycol)-based hydrogels following published procedures.<sup>27, 35-36</sup> Specifically, we mixed the 4-arm-PEG-NH<sub>2</sub> in borate buffer at pH 8.6 with NHS-PEG-NHS, which contains the NHS-esters, in phosphate buffered saline (PBS 1x) buffer at pH 7.4 to form the amide-bond linked network (Figure 2A). We maintained the molar ratio of amine to the NHS at 1:1, and varied the total concentration of the polymer in solution from 5, 10, 25, to 50 wt%.

First, we evaluated the pore size of the lyophilized hydrogels through scanning electron microscopy. Pore sizes are  $14.4 \pm 1.7$ ,  $11.8 \pm 1.0$ ,  $10.3 \pm 1.4$ , and  $9.7 \pm 0.4$  microns for the 5 wt%, 10 wt%, 25 wt%, and 50 wt% hydrogels, respectively (Figures 2B, C, D, and E). The pore size slightly decreases with increasing polymer weight percents. Additionally, we examined the morphology of the dried and hydrated hydrogel samples using atomic force microscopy (AFM). AFM analysis of the dried hydrogel reveals a surface roughness of  $131 \pm 21$  nm, defined by the root-mean-square value of the height (Figure 2F). Upon hydration, the hydrogel exhibits a more homogeneous and smoother surface, with a decreased surface roughness of  $19 \pm 7$  nm (Figure 2G). Next, we assessed the swelling characteristics of the hydrogels prior to integration with QD-based FRET sensor. After exposure to PBS 1x, the hydrogels swell and reach equilibrium by day 4 for all polymer weight percents evaluated, while maintaining their structural integrity (Figure 2H). The swelling degree positively correlates with the initial polymer content (percent by weight). The diffusion of small molecules (water, in this case) through the hydrogel translates to its capacity to absorb and release analyte of interest, which is essential for biosensor functionality. As it is important that the hydrogels possess integrity, we evaluated the viscoelastic properties and mechanical strength of the hydrogels. First, we determined the linear viscoelastic region (LVR) by performing the oscillatory stress sweep (Figures S2, 3, 4, 5A). Next, we measured the frequency sweep at a controlled oscillatory stress of 1 Pa chosen from the LVR (Figures S2, 3, 4, 5C). Across the frequencies tested, the hydrogels exhibit solid-like behavior (i.e.,  $G' > G''$ ). Lastly, we performed the same experiments on swelled hydrogels (Figures S2, 3, 4, 5B and D). The storage ( $G'$ ) and loss ( $G''$ ) moduli positively correlate with the increase in weight percent of the hydrogels (Figures 2I, J, K, and L). At the swelling equilibrium, the  $G'$  values decrease significantly for all four hydrogels compared to the as prepared hydrogel prior

to swelling. Taken together, the ability of the hydrogel to hold water translates to its capacity to absorb analyte solution, and maintain an aqueous environment for the sensor components. For example, after exposure to PBS 1x, the 10 wt % hydrogel swells 221%, reaching equilibrium, while maintaining its integrity and mechanical strength to allow for use and easy handling. With tunable mechanical properties and structural integrity, these hydrogels hold promise for QD-based FRET sensor integration.

**3.2. Diffusion Studies.** The QD-FRET sensor is based on an affinity-based biorecognition element.<sup>8</sup> This sensing framework requires binding of TF to its cognate DNA site in the absence of analyte and subsequent unbinding in the presence of analyte. As such, the immobilization matrix must successfully entrap the QD-based FRET (i.e., quantum dot - transcription factor DNA assembly) sensor, while allowing transport of the analyte and subsequent binding to the TF-DNA complex.

First, we characterized the diffusion of biomolecules within the hydrogels by fluorescence recovery after photobleaching (FRAP). Given a molecule's diffusion coefficient is affected by its molecular weight, we estimated the diffusion behavior of progesterone using fluorescein, a molecule with similar molecular weight (fluorescein MW = 332.31 g/mol, progesterone MW = 314.46 g/mol). Fluorescein homogeneously distributes within the hydrogel and, upon photobleaching, within a region of interest the fluorescence recovers as a function of time (Figure S6). The measured diffusion coefficient negatively correlates with hydrogel weight percent (Figure 3A). With increasing hydrogel weight percent, the mobility of fluorescein is statistically significantly hindered. We repeated the experiments with Cy5 labeled DNA and the fluorophore labeled TF, SRTF1. Analogously, the Cy5 labeled DNA and SRTF1 exhibit decreasing diffusion coefficients with increasing hydrogel weight percent (descending correlation; Figure 3B and C). The entanglement and crosslinking of the hydrogel, which is tunable by varying polymer weight percent, influences the diffusional coefficients of molecules and biomacromolecules, consistent with previous reports.<sup>37-39</sup>

Next, we measured the diffusion coefficients of QD and QD-TF under semi-infinite diffusion condition. Unlike the experiments above, the QD is difficult to photobleach due to its photostability, rendering techniques like FRAP unsuitable for the diffusion coefficient measurement.<sup>40</sup> Thus, we directly monitored the mass transfer by acquiring vertical cross section images of hydrogel with QD (or QD-TF) as it moved through the hydrogel (Figure S7). Fitting the intensity profiles of the cross-sectional images to Fick's second law yields the diffusion coefficient. The magnitude of the QD diffusion coefficient declines with respect to increasing hydrogel weight percent (Figure 3D). Conjugation of TF with QD further attenuates the diffusion coefficient of QD-TF (Figure 3E).

Lastly, we compared all of the diffusion coefficients of the species or sensor constituents in different wt% hydrogels. In 5 wt% hydrogel, the small molecule fluorescein possesses a diffusion coefficient > 25-fold higher than the other sensor constituents (Figure S8). In 10 wt% hydrogel, the fluorescein possesses a diffusion coefficient > 60-fold higher than the other sensor constituents (Figure 3F). Additionally, the absolute diffusion coefficients of fluorescein and the sensor constituents are decreased compared to the values measured in the 5 wt% hydrogel. The significantly faster diffusion of

fluorescein indicates the feasibility of analyte molecules diffusing in and out of the hydrogel network while the sensor components remain entrapped. In the 25 wt% hydrogel, the fluorescein possesses a diffusion coefficient > 135-fold higher than the other sensor constituents, but the overall significantly hindered diffusion implies undesirable longer diffusion time to add or remove analyte (to be describe in Section 3.4). Collectively, these results suggest that the integration of QD-based FRET sensors into the hydrogel will: 1) allow the progesterone molecules to diffuse in and out easily; and, 2) hinder the diffusion of the quantum dot-TF conjugate (FRET donor) and Cy5 fluorophore modified DNA (FRET acceptor), thus minimizing the undesirable leakage of sensor constituents.<sup>18, 41</sup> Consequently, we evaluated the 10 wt% hydrogels throughout the course of the remaining studies.

**3.3. Sensor Performance in Hydrogel.** The discussion of the hydrogel-loaded QD-based FRET sensor is separated into three stages. First, we assembled the sensor constituents at a molar ratio of QD/TF/DNA = 1/4/18. Specifically, the FRET donor is the TF-conjugated QD, and the DNA oligonucleotide is decorated with fluorophores as FRET acceptor. Next, we dissolved the two PEGylated macromonomers in the sensor solution and formed the hydrogel *in situ* to entrap sensors over approximately 30 minutes. When the TFs bind their cognate DNA binding sites in the absence of progesterone, the donors and acceptors are sufficiently close to enable energy transfer. Lastly, we subjected the QD-based FRET sensors embedded in the hydrogel to a solution of progesterone overnight. Upon progesterone binding to the transcription factor, the affinity of the TF for its cognate DNA reduces by several orders of magnitude;<sup>8</sup> the resultant unbinding of the TF and DNA increases the distance between the associated FRET donors and acceptor, producing a decrease in the fluorescence of the acceptor ( $F_A$ ) and increase in the fluorescence of the donor ( $F_D$ ) (Figure S10). The sensor output is the normalized ratio of the integrated acceptor emission to the integrated donor emission ( $F_A/F_D$ ). A four-parameter logistic function analysis of progesterone binding as a function of concentration reveals a dose-dependent decrease in sensor output (Figure 4). The constructed sensor permits the measurement of progesterone from 78 to 632 nM. The  $EC_{50}$  and LOD values of the hydrogel imbedded sensor are 223 nM and 55 nM, respectively, and comparable to solution-based counterpart (Table S2). This solution-based progesterone TF-DNA sensor does not respond to cholesterol, estrogen, 5 $\beta$ -Pregnane-3 $\alpha$ ,20- $\alpha$ -diol, or 5 $\beta$ -Pregnane-3 $\alpha$ ,20- $\alpha$ -diol-glucuronide while affords a 10% and 13% response to aldosterone and cortisol, respectively, indicating it is reasonably selective for progesterone.<sup>8</sup> Additionally the progesterone TF-DNA sensor performs in artificial urine<sup>8</sup>.

Previously, QDs have been extensively employed in the context of solution-based sensors;<sup>42-45</sup> however, only recently has it been reported that hydrogel-embedded QDs (semiconductor quantum dots,<sup>20, 46-52</sup> carbon dots,<sup>53</sup> and graphene quantum dots)<sup>54-55</sup> elicit effective analytical responses, similar to the results obtained herein. In addition, several studies report the successful integration of solution-based QD-based sensors into microfluidic lab-on-a-chip systems<sup>56-57</sup> or microcapsules<sup>58</sup> supporting the continued development of these types of sensors for real world applications.

**3.4. Repeated Progesterone Sensing.** One can envision two sensing formats for a progesterone measuring device: single or repeat use. The latter is more demanding

from a device development standpoint as repeated use requires the TF/DNA complex to remain in close proximity for assembly and dis-assembly while the analyte must be able to diffuse in and out. To assess our system's capability to perform multiple measurements, we subjected hydrogel-embedded QD-based FRET sensors to cycles of progesterone exposure and removal between measurements. The sensor output – the change in FRET - is derived from deconvolved fluorescent spectra and was fit to a four-parameter logistic function as described above (Figure 5). After regeneration, the sensor displays a dose-dependent response with an  $EC_{50}$  of 159 nM and  $R^2 = 0.76$ , comparable to first-time sensing ( $EC_{50} = 223$  nM, and  $R^2 = 0.98$ ). Notably, the relatively small differences in the dose-dependent sensor response in the first and second measurements (not statistically significantly different for most cases) shows the potential for transcription factor-based biosensors in repeated sensing applications without the need for additional procedures to reform the nucleic acid - transcription factor complex for subsequent sensing (Figure S11). Greater variability is observed between measurements after dialysis, and this is likely due to limitations in the ability of dialysis to completely remove progesterone. Finally, we note that the relatively long time for dialysis to remove progesterone is a limitation for repeated measurements but not for single use measurements, and this will need to be overcome in future designs.

The sensor consists of a transcription factor (SRTF1) linked to a core/shell/shell QD and a Cy5 fluorophore labeled DNA, with an immobilization matrix composed of a hydrogel. From a sensor performance perspective, the LOD is 55 nM and the device is suitable for clinically relevant sample analysis.<sup>59</sup> Such a QD-based FRET sensor is also advantageous as it enables repeated analyte measurements. By contrast, immunoassays are considered effectively irreversible, stemming from fixation of antibody-analyte binding.<sup>60</sup> The regeneration of immunosensors pose significant barriers if prolonged use is desired.<sup>61</sup> As such, this study represents a first step toward assessing the potential of using transcription factor-based sensors in the context of single, or repeat use for practical applications.

From a sensor design perspective, the potential versatility of our transcription factor-based sensor stems from diverse biorecognition elements mined from the nature. In bacteria, TFs natively sense and react to a wide variety of stimuli and transduce a measurable transcriptional response – an alteration in reporter gene expressions.<sup>62</sup> Previously, TFs have been used as *in vivo* biosensors with the advances in synthetic biology, yet hampered by slow response time and biosafety concerns for practical applications as the genetically re-programed bacteria itself is the biosensor;<sup>7, 63-67</sup> however, only recently has it been deployed *in vitro*, similar to this study.<sup>68-69</sup> Although known, the lack of characterized TFs prohibits the evaluation, optimization, and prototyping of next generation biosensors to wide variety analytes. To address this problem, a recent genomics-to-sensor approach represents a potential paradigm shift in biosensor development, which identifies and harvests biosensing elements from high-throughput microbial genomic sequencing.<sup>2, 8</sup> Metagenomic sequence mining continues to expand the repertoire of natural sensors<sup>3-4</sup>, and tap directly into this rich diversity, being a first line solution to the lack of biosensors. Finally, due to modular design of our sensor system, developing a wealth of new affinity-based FRET sensor becomes feasible, capitalizing on TFs with novel analyte specificities.



#### 4. CONCLUSIONS

We describe a hydrogel-immobilized biosensor based on allosteric transcription factor-DNA binding. Specifically, this Förster resonance energy transfer sensor comprises a quantum dot-TF conjugate (FRET donor), a Cy5 fluorophore modified DNA (FRET acceptor), and a hydrogel immobilization matrix. Upon progesterone binding, the QD-TF assembly dissociates, resulting in a decrease in ratiometric fluorescent output as the TF and QD are now no longer in close proximity. The key elements of this sensor include: 1) TF-DNA coupling as a recognition/ transduction element; 2) fluorescent nanoparticles, QDs, that resist photobleaching and efficiently transfer energy to the fluorophore labeled DNA; 3) a hydrogel immobilization matrix, polymerized *in situ* to entrap sensors, while enabling analyte diffusion and binding; and, 4) repeated progesterone sensing through capitalizing on the reversible interactions associated between the transcription factor and DNA. We foresee a convergence of high-throughput microbial genomic sequencing to identify new biorecognition elements, and artificial intelligence algorithms to acquire and process sensor signals, as well as immobilization and integration into wearable microfluidic devices advancing digital health.

## ASSOCIATED CONTENT

### Supporting Information.

The Supporting Information is available free of charge on the ACS Publications website at DOI: xxx

Figure S1.  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR of 4-arm-PEG-NH<sub>2</sub> and NHS-PEG-NHS; Figure S2. Mechanical properties of 5 % hydrogel; Figure S3. Mechanical properties of 10 % hydrogel; Figure S4. Mechanical properties of 25 % hydrogel; Figure S5. Mechanical properties of 50 % hydrogel; Figure S6. Diffusion coefficient measurement of fluorescein in 10 wt% hydrogel; Figure S7. Diffusion of QD in 5 % hydrogel; Figure S8. Diffusion coefficients of the species or sensor constituents in different wt% hydrogels. Figure S9. Absorption (dashed) and fluorescent emission (solid) of QDs (yellow,  $\lambda_{\text{ex}} = 400$  nm) and Cy5-DNA (red,  $\lambda_{\text{ex}} = 590$  nm) in 10 wt % hydrogel. Figure S10. Hydrogel embedded sensor's response to analyte titration. Figure S11. Reversibility of the sensor.

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### Conflict of Interest

The authors have submitted a patent application on the biosensor technology.

### Author Contributions

M. Chen and MWG conceptualized and designed the overall study. MC conducted all experiments and analyzed the data. CG, TN, and M. Chern assisted with QD preparation. PS helped collect spectral data. NV conducted AFM-related studies and analyzed the data. YF assisted with diffusion measurement. RCB provided the protein. KC analyzed NMR data. The manuscript was written by M. Chen and MWG with contributions from all authors. All authors have given approval to the final version of the manuscript.

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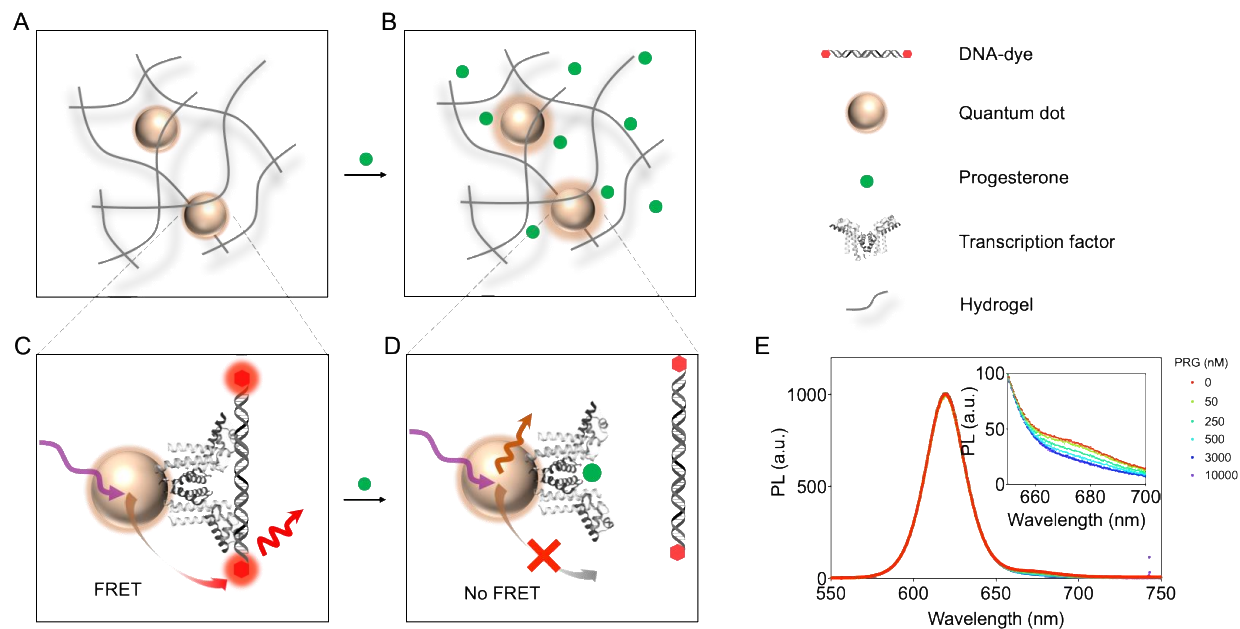


Figure 1. QD-based FRET sensor immobilized in hydrogel. (A) and (B) Schematic illustration of the progesterone diffusion into hydrogel. (C) and (D) FRET-based sensor utilizing TF-DNA binding mechanism. When progesterone binds to the TF, the affinity between the TF and the DNA reduces. The resulting dissociation of the TF-DNA complex produces a decreased FRET signal. (E) Representative spectra of sensor response to progesterone. A selection of the analyte concentrations is plotted for visual clarity. Zoomed in figure is shown in the inset.

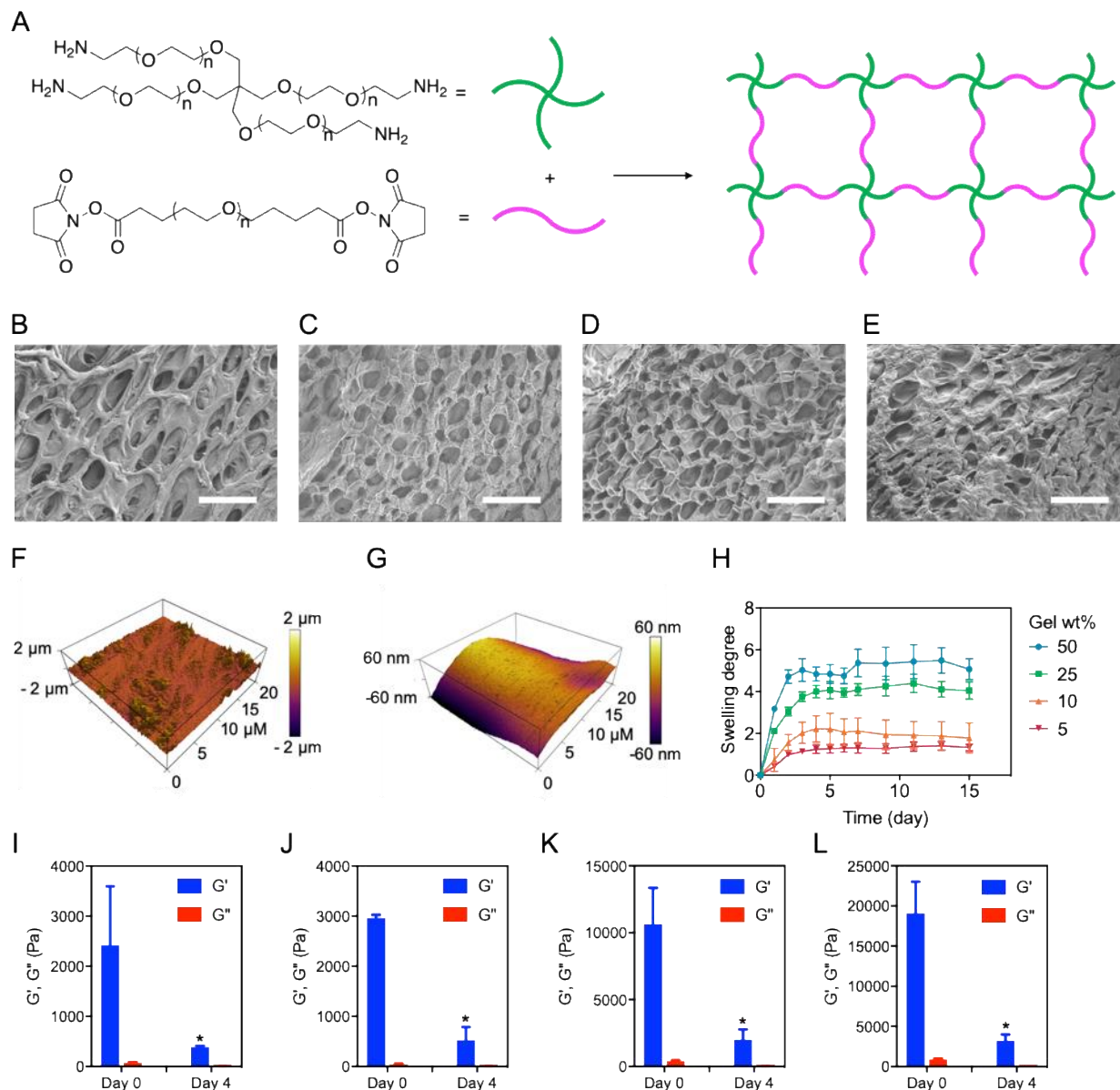


Figure 2. Hydrogel precursors, formation, and swelling. (A) Chemical structures of the hydrogel forming components: 4-arm-PEG-NH<sub>2</sub> and NHS-PEG-NHS. Hydrogel formation proceeds via a reaction between NHS ester (*N*-hydroxysuccinimide ester) and primary amine, producing a 3D network. The crosslinked network enables encapsulation of the QD-based FRET sensor. Scanning electron microscopy images of (B) 5 wt%, (C) 10 wt%, (D) 25 wt%, and (E) 50 wt% hydrogels, scale bar is 50 μm. Atomic force microscopy image of hydrogel (F) before and (G) after hydration. (H) Swelling degree of the hydrogel samples at 5, 10, 25, and 50 wt%, stored in PBS at 20 °C as a function of time. Storage and loss moduli of (I) 5 wt%, (J) 10 wt%, (K) 25 wt%, and (L) 50 wt% hydrogels with oscillatory stress of 1Pa at the frequency of 1Hz before and after swelling. \*Statistical significance (95% confidence) for mechanical strength relative to respective Day 0 data.

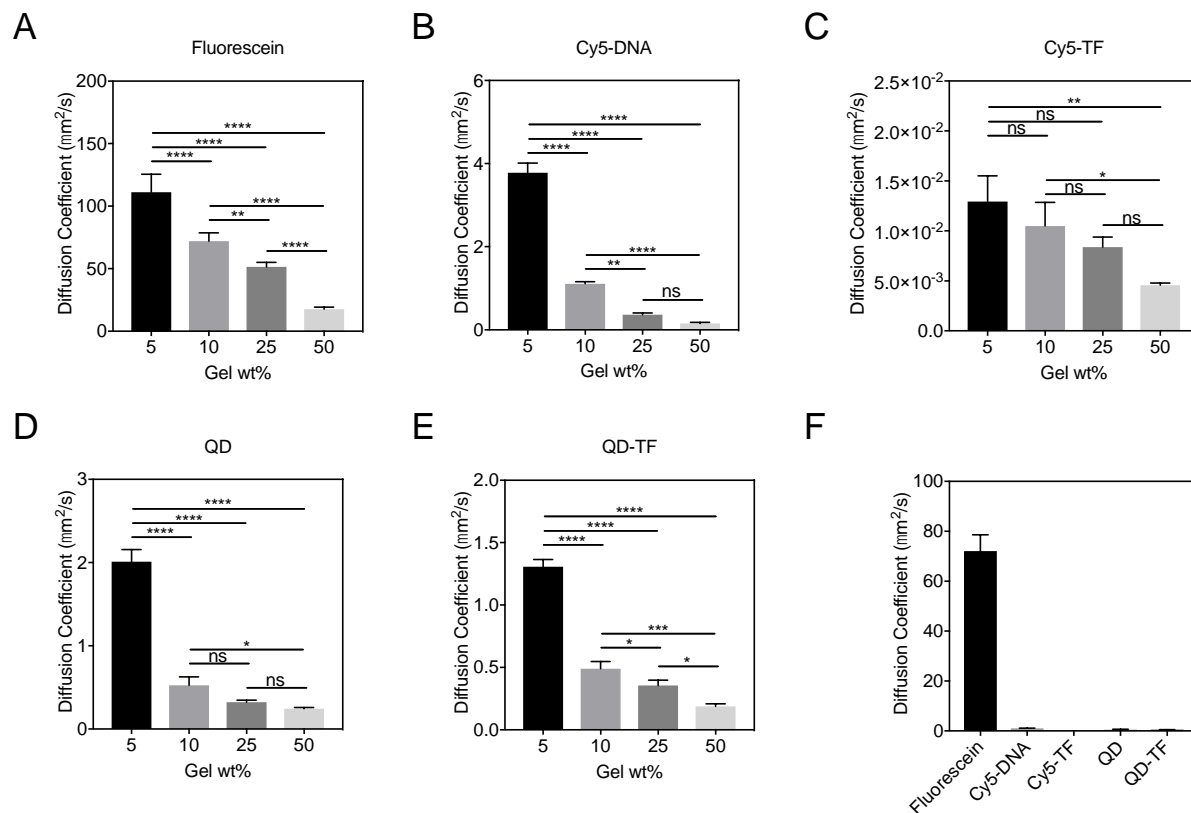
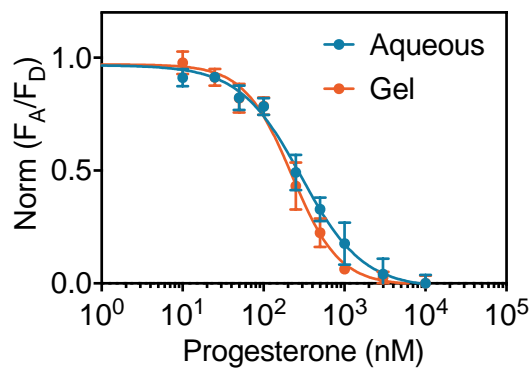
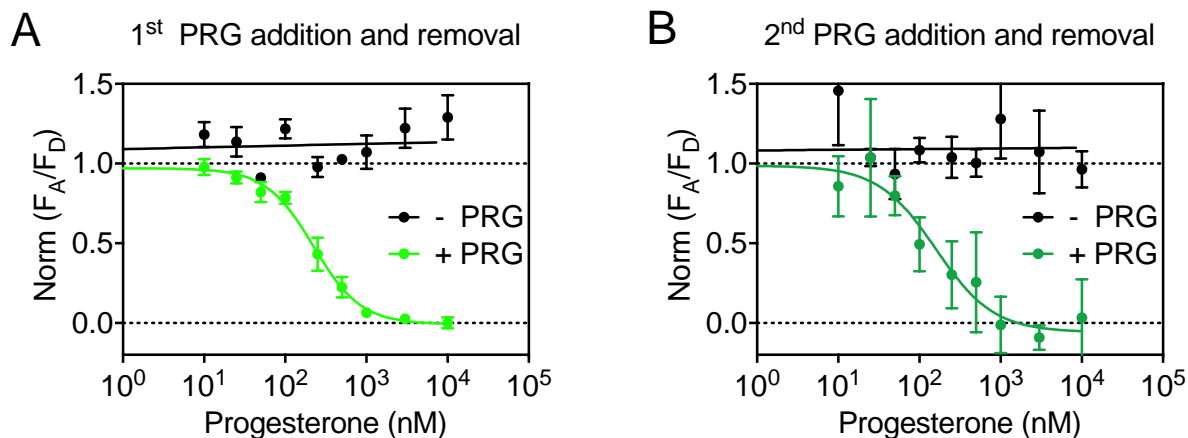


Figure 3. Diffusion coefficients of QD-based FRET sensor constituents. Diffusion coefficients of (A) fluorescein, (B) Cy5-DNA, and (C) Cy5-TF, determined by fluorescence recovery after photobleaching (FRAP). Diffusion coefficients of (D) QD and (E) QD-TF, determined by monitoring 100 nM QD or QD-TF diffusion into hydrogel. (F) Diffusion coefficients of sensor constituents in 10 wt% hydrogel. N=3 \*  $P \leq 0.05$ . \*\*  $P \leq 0.01$ . \*\*\*  $P \leq 0.001$ . \*\*\*\*  $P \leq 0.0001$ . ns, not significant.



**Figure 4.** Dose-response curves for progesterone sensors in aqueous solution and in 10 wt% hydrogel immobilization matrix, respectively.  $F_A/F_D$  at  $[PRG] = 0$  nM is normalized to 1,  $F_A/F_D$  at  $[PRG] = 10,000$  nM is normalized to 0, and linear interpolation is applied for concentrations in between.



**Figure 5.** Reversibility of the progesterone sensors immobilized in hydrogel. (A) Sensor response with first cycle of progesterone exposure and removal. (B) Sensor response with second cycle of progesterone exposure and removal. With repeated cycles of progesterone exposure (+), dose-response curves are generated (green data points: light green points for first PRG addition, and dark green points for second PRG addition). After PRG removal (-), sensor outputs are plotted at corresponding concentrations to which the same samples were exposed (black data points). With PRG addition,  $F_A/F_D$  at  $[PRG] = 0$  nM is normalized to 1,  $F_A/F_D$  at  $[PRG] = 10,000$  nM is normalized to 0, and linear interpolation is applied for concentrations in between. After PRG removal,  $F_A/F_D$  is scaled, where  $F_A/F_D$  at  $[PRG] = 0$  nM is normalized to 1.



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