Supporting Information

DNA-Coated Gold Nanoparticles for the Detection of mRNA in Live *Hydra Vulgaris* Animals

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S-I General

Reagents used throughout this study were purchased from the following suppliers: Trisodium citrate, agarose, sodium tetrachloroaurate (III) dihydrate and Bis(p-sulfonatophenyl)phenyl phosphine dehydrate dipotassium salt (BSPP) were purchased from Sigma Aldrich whilst all oligonucleotides were synthesized using a standard phosphoramidite solid phase synthesis by ATD Bio Ltd.

UV-visible spectra were acquired on a UV Cary 100 UV-Vis spectrophotometer over the range of 400 to 800 nm whereas fluorescence spectra were collected in a low volume black quartz cuvette on a Carry eclipse fluorescence spectrophotometer. Zeta potential analysis was performed on a Malvern Zetasizer Nano ZS and oligonucleotide absorbance was assessed on a DeNovix DS-11 Spectrophotometer.

S-II Supplementary experimental data

a. Anatomy of *Hydra vulgaris*

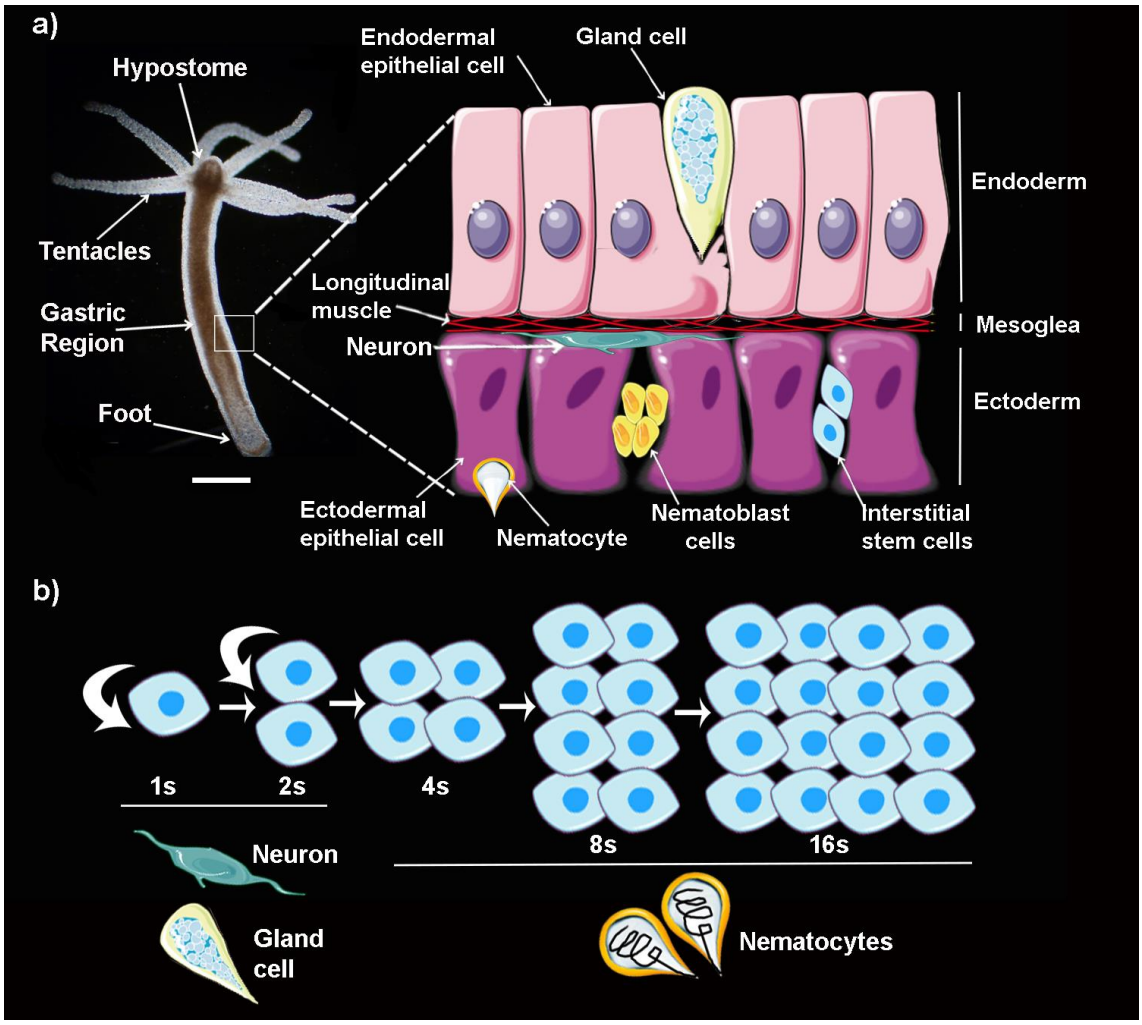

Figure S1. Anatomy of *Hydra* and differentiation pathways of interstitial cells. a) Microscopy image of living *Hydra*. The polyp is composed of a bilayered body column, with a ring of tentacles around the hypostome at the apical region and a foot at the basal region. The cellular bilayer is composed of an outer ectoderm and an inner endoderm, which are separated by an extracellular matrix, the mesoglea. Interstitial stem cells and nematoblasts are allocated between ectodermal epithelial cells, while gland cells are located between endodermal epithelial cells. Neurons can be found both in the ectoderm and endoderm. b) Schematic representation of the multiple differentiation pathways of the interstitial stem cells. Interstitial stem cells include multipotent stem cells (1s) and committed stem cells (2s) that give rise to four classes of differentiated cells (nematoblasts, neurons, secretory cells and gametes). Nematocytes are derived from committed cells after different divisions, which give rise to nests of 4, 8 or 16 cells (nematoblasts). Scale bar is 500 µm.

b. Supplementary microscopy data

b. i. Lower magnification microscopy images

Lower magnification images were acquired showing the complete *Hydra* body after incubation with Hymyc1–nanoprobes (that detect only Hymyc1 mRNA), scramble–nanoprobes (that do not detect any mRNA) and general mRNA–nanoprobes (that detect all mRNAs). The Hymyc1-nanoprobes detected Hymyc1 mRNA, which was localized to the main body of hydras while the general mRNA-nanoprobes (gmRNA-nanoprobes) detected all mRNAs distributed everywhere in the animal.

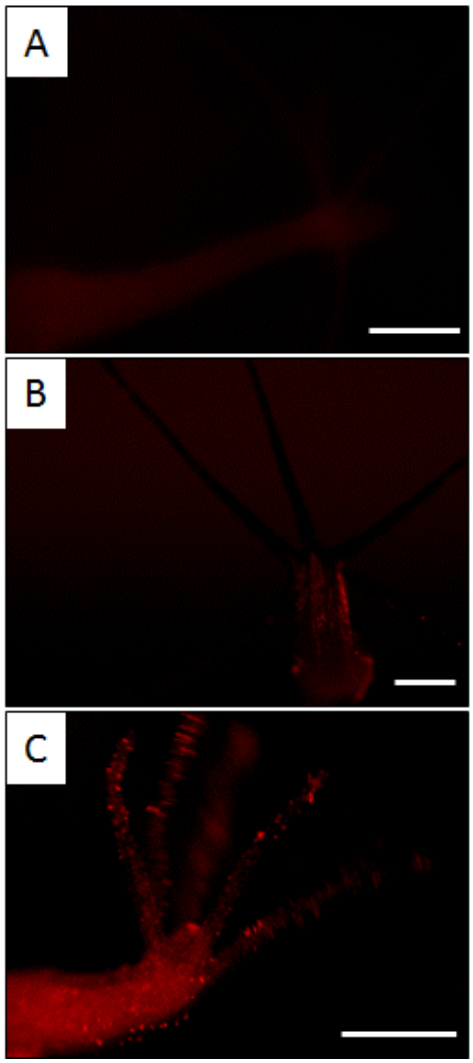

Figure S2. Fluorescence microscopy images of live *Hydra* incubated with scramble–nanoprobes (A), Hymyc1–nanoprobes (B) and gmRNA–nanoprobes (C). Scale bar: 500 µm.

S-III Oligonucleotide sequences

Oligonucleotide sequences used, including 'sense' and 'flare' strands for all gold nanoparticle (AuNP) probes, are presented in the following table. These include sequences specific for the detection of Hymyc1 mRNA (Hymyc1 – nanoprobe) as well as sequences specifically designed as a negative (scramble – nanoprobe) and positive (gmRNA – nanoprobe) control.

Table S1: Sense and flare strand oligonucleotide sequences presented from 5' to 3' where X = thiol modifier 6 S-S (CPG resin from Glen Research).

Hymyc1 – nanoprobe	Sense strand	**FAM** – TTCTTGACGCGTGAGCATCTTAAAAAAAA – X
	Flare strand	**Cy3** – AAGATGCTCA
Scramble – nanoprobe	Sense strand	**FAM** – TCGAAGTATTCCGCGTACGTTAAAAAAAA – X
	Flare strand	**Cy3** – AACGTACGCG
gmRNA – nanoprobe	Sense strand	X – AAACGGGCTTTTTTTTTTTTTTTTTTTTTTTTTTT – **FAM**
	Flare strand	AAAAAAAAAAAAGCCC – **Cy3**

S-IV Synthesis of oligonucleotide sequences

Oligonucleotides were synthesized on an Applied Biosystems 394 automated DNA/RNA synthesizer using a standard 1.0 μmole phosphoramidite cycle of acid-catalyzed detritylation, coupling, capping, and iodine oxidation. Stepwise coupling efficiencies and overall yields were determined by the automated trityl cation conductivity monitoring facility and was > 98.0 %. Standard DNA phosphoramidites. Solid supports (3'-Thiol-Modifier C3 S-S CPG, item number: 2361, 3'-(6-FAM)-CPG, item number: 2366 and 3'-Cyanine-3 SynBase™ CPG 1000/110, item number: 2412) and modified phosphoramidites (Thiol-Modifier C6 S-S CE Phosphoramidite, item number: 2126, Cyanine-3-CE Phosphoramidite (Cyanine 540), item number: 2520 and

5'-Fluorescein-CE Phosphoramidite (6-FAM), item number: 2134) were purchased from Link Technologies Ltd. Additional reagents were purchased from Applied Biosystems Ltd. All β-cyanoethyl phosphoramidite monomers were dissolved in anhydrous acetonitrile to a concentration of 0.1 M immediately prior to use with coupling time of 50 s for normal A, G, C, and T monomers and was extended to 600 s for modified monomers. Cleavage and deprotection were achieved by exposure to concentrated aqueous ammonia solution for 60 min at room temperature followed by heating in a sealed tube for 5 h at 55 °C. Purification was carried out by reversed-phase HPLC on a Gilson system using a Brownlee Aquapore column (C8, 8 mm x 250 mm, 300 Å pore) with a gradient of acetonitrile in triethylammonium bicarbonate (TEAB) increasing from 0 % to 50 % buffer B over 30 min with a flow rate of 4 mL/min (buffer A: 0.1 M triethylammonium bicarbonate, pH 7.0, buffer B: 0.1 M triethylammonium bicarbonate, pH 7.0 with 50% acetonitrile). Elution was monitored by ultraviolet absorption at 298 nm. After HPLC purification, oligonucleotides was freeze dried then dissolved in water without the need for desalting. All Purified oligonucleotides were characterised by electrospray mass spectrometry. Mass spectra of oligonucleotides were recorded either using a BrukermicrOTOFTM II focus ESI-TOF MS instrument in ES- mode or a XEVO G2-QTOF MS instrument in ES- mode. Data were processed using MaxEnt and in all cases confirmed the integrity of the sequences.

S-V DNA-coated AuNP characterization

DNA-coated AuNP probes were thoroughly characterized using a number of different techniques in order to assess the efficiency of AuNP functionalisation with a monolayer of 'sense' strands as well as the efficiency of 'flare' strand hybridization

a. AuNP surface functionalization with oligonucleotide 'sense' strands

Successful AuNP functionalization with oligonucleotide 'sense' strands was qualitatively and quantitatively assessed using the following techniques.

a. i. UV – Vis spectroscopy

Following functionalization and purification UV-Vis spectra of DNA–coated AuNPs were acquired and compared to BSPP functionalized AuNPs as shown in **Figure S1**.

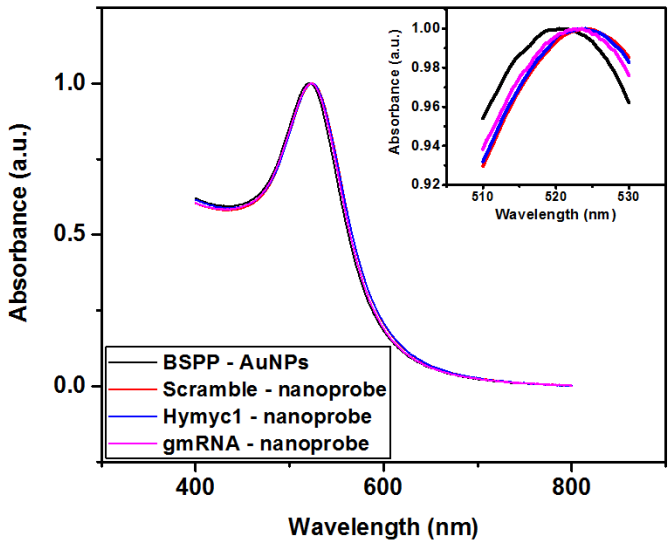

Figure S3. Normalized Uv-Vis spectra of oligonucleotide functionalized AuNPs in comparison to BSPP coated AuNP. After functionalization and purification a small red-shift in the absorbance peak maximum was observed, which was attributed to the oligonucleotide coating on the AuNP surface.

a. ii. Gel electrophoresis

AuNPs functionalized with a monolayer of oligonucleotides were also analyzed *via* gel electrophoresis as a method to qualitatively determine successful 'sense' strand attachment. Samples were run in a 1.75 % agarose gel with 0.5 x TBE buffer (pH 8) at 10 V/cm until distinct bands were visible (gel presented in **Figure S4** was run for 30 minutes)

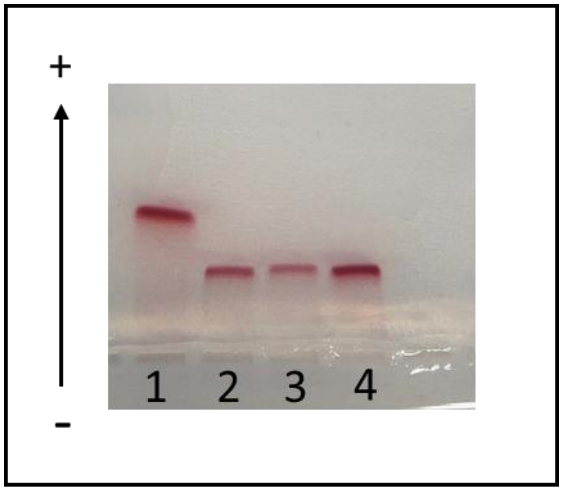

Figure S4. DNA-coated AuNPs analyzed *via* gel electrophoresis. **Lane 1:** AuNPs stabilized with BSPP, **Lane 2:** Hymyc1– nanobrobes **Lane 3:** scramble – nanobrobes and **Lane 4:** gmRNA – nanoprobes. AuNPs functionalized with a monolayer of oligonucleotides showed a clear band delay compared to BSPP – AuNPs, which was attributed to an increase in size thus demonstrating successful oligonucleotide attachment. Furthermore, bands in Lane 2, 3 and 4 demonstrated a similar electrophoretic mobility indicating that the degree of surface functionalization presents no significant difference.

a. iii. Zeta potential

The net surface charge of functionalized AuNPs was also assessed *via* zeta potential measurements and it is presented in **Table S2**.

Table S2: Net surface charge of oligonucleotide modified AuNPs

Type of nanoprobe	Surface Charge (mV)
Hymyc1 – nanoprobes	-29.7 ± 2.9
Scramble – nanoprobes	-29.7 ± 5.0
gmRNA – nanoprobes	-27.9 ± 3.7

a. vi. Quantitative analysis of 'sense' strand loading

AuNP functionalization was also quantitatively assessed in order to determine the exact number of oligonucleotide strands attached to the AuNP surface after purification. To achieve this the AuNP core was dissolved using a previously described protocol and the oligonucleotide concentration was determined using specific settings for ssDNA on the Denovix spectrophotometer.[1]

Table S3: Determination of 'sense' strand oligonucleotide loading for each type of nanoprobe

		Hymyc1 – nanoprobe		
Measurement	ng/µL	Concentration (µM)	No of moles (pmol)	Number of 'sense'strands/ AuNP
1	1.668	0.142	213.7	106.8
2	1.716	0.147	220.0	110.0
3	1.771	0.151	227.2	113.6
SEM	0.029	0.003	3.2	**1.9**
Average	1.718	0.147	220.3	**110.1**
		gmRNA – nanoprobe		
1	1.917	0.157	236.3	118.1
2	1.962	0.161	241.6	120.8
3	1.810	0.148	222.9	111.4
SEM	0.045	0.004	5.6	**2.8**
Average	1.896	0.155	233.6	**116.8**
		Scramble – nanoprobe		
1	1.590	0.133	200.2	133.4
2	1.245	0.104	156.6	104.4
3	1.271	0.106	159.9	106.6
SEM	0.111	0.009	14.0	**9.3**
Average	1.369	0.115	172.2	**114.8**

b. Efficiency of 'flare' strand hybridization

b. i. Melting curves

Melting curves of the three types of probes were run to determine the melting temperature of the 'sense'/ 'flare' strand duplex and to ensure that it is above 37 °C. Nanoprobes (2.5 nM, 200 µL) were gradually heated from 25 °C to 75 °C at a rate of 1

°C/ minute. At low temperature the ‘flare’ strand remains bound to the ‘sense’ strand. A further increase in temperature allows for the de-hybridization of the duplex, which is observed as an increase in the fluorescence signal of the dye-modified (Cy3) flare strand (excitation: 544 nm, emission 562 nm).

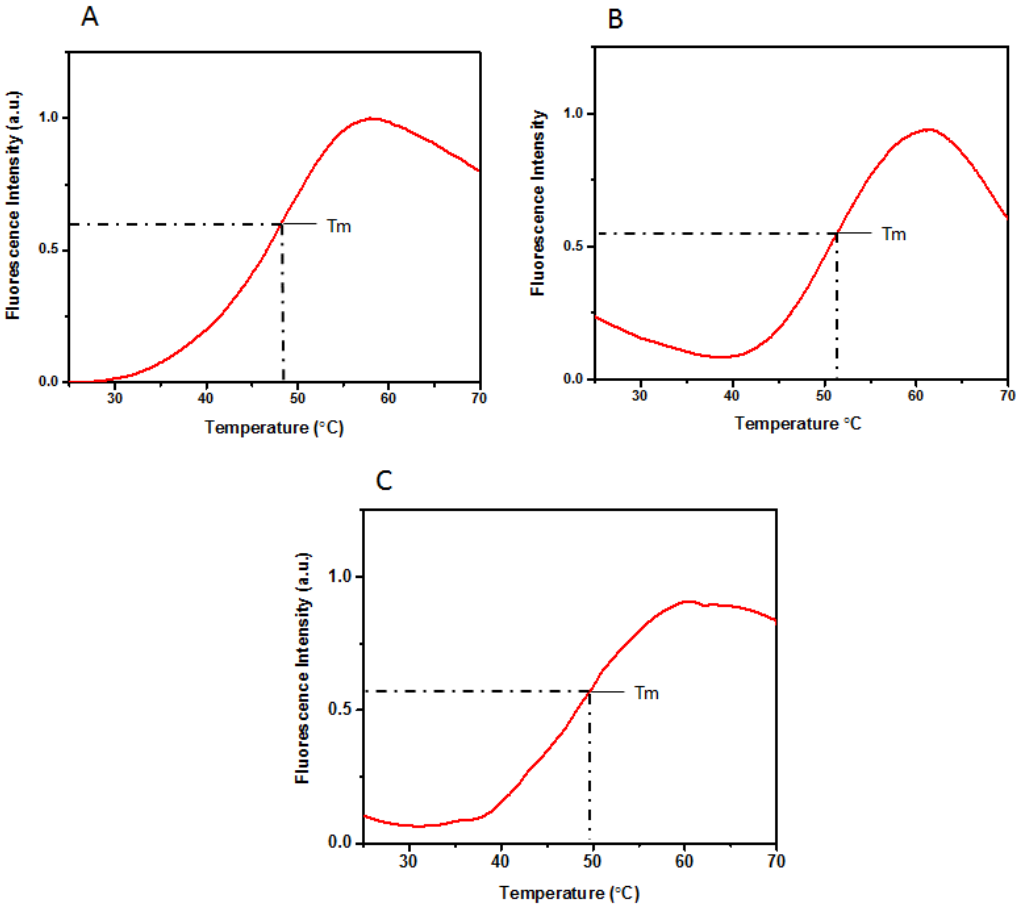

Figure S5. Fluorescence melting analysis of successful sense/flare hybridization for hymyc1 (A), scramble (B) and gmRNA (C) nanoprobes. Nanoprobes were gradually heated to 80 °C whilst monitoring the fluorescence output of the Cy3 modified ‘flare’ strand.

b. ii. Quantitative analysis of ‘flare’ strand hybridization

In order to quantitatively assess the number of ‘sense’/ ‘flare’ duplexes per AuNP probe, a sample of each probe (5 nM, 200 µL) was heated to 70 °C for 10 minutes to allow for ‘flare’ strand de-hybridization. The sample was immediately centrifuged (16,400 rpm, 20 minutes) and the supernatant was collected and analysed on the

Denovix spectrophotometer in order to calculate the number of duplexes for each probe as presented in **Table S4**.

Table S4: Determination of oligonucleotide 'flare' strand loading

		Hymyc1 – nanoprobe		
Measurement	ng/μL	Concentration (μM)	No of moles (pmol)	Number of 'flare'strands/ AuNP
1	1.228	0.305	61.1	61.1
2	1.094	0.273	54.5	54.5
3	1.055	0.263	52.6	52.6
SEM	0.052	0.013	2.6	**2.6**
Average	1.126	0.280	56.1	**56.1**
		gmRNA – nanoprobe		
1	2.006	0.272	54.4	54.4
2	2.244	0.304	60.9	60.9
3	2.377	0.322	64.4	64.4
SEM	0.108	0.015	2.9	**2.9**
Average	2.209	0.299	59.9	**59.9**
		Scramble – nanoprobe		
1	1.117	0.290	58.0	58.0
2	1.188	0.310	61.8	61.8
3	1.365	0.354	70.9	70.9
SEM	0.074	0.019	3.8	**3.8**
Average	1.223	0.318	63.6	**63.6**

REFERENCES

1. Kyriazi, M. E.; Giust, D.; El-Sagheer, A. H.; Lackie, P. M.; Muskens, O. L.; Brown, T.; Kanaras, A. G., Multiplexed mRNA Sensing and Combinatorial-Targeted Drug Delivery Using DNA-Gold Nanoparticle Dimers. *ACS Nano* **2018,** *12*, 3333-3340.