Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information for

Production and Electrical Characterization of the Reflectin A2 Isoform from *Doryteuthis (Loligo) pealeii*

David D. Ordinario[1,†], Long Phan[1,†], Ward G. Walkup IV[1,2,†], Yegor Van Dyke[1], Erica M. Leung[1], Michael Nguyen[1], Amanda G. Smith[1], Justin Kerr[1], Mahan Naeim[1], Ioannis Kymissis[3], Alon A. Gorodetsky[1,4,*]

[1]Department of Chemical Engineering and Materials Science, University of California, Irvine, Irvine, CA 92697, USA

[2]Current Address: Department of Biology and Biological Engineering, California Institute of Technology, Pasadena, CA 91125, USA

[3]Department of Electrical Engineering, Columbia University, New York, NY 10027, USA

[4]Department of Chemistry, University of California, Irvine, Irvine, CA 92697, USA

†Denotes equal contribution

[*]To whom correspondence may be addressed. Email: alon.gorodetsky@uci.edu

Experimental Methods and Procedures:

(I) ***Expression and Purification of Reflectin A2.*** Histidine-tagged wild type reflectin A2 (RfA2) was expressed and purified *via* a protocol that was modified from an analogous one previously reported for wild type reflectin A1 (RfA1)[S1,S2]. In brief, an *E. coli* codon optimized gene coding for histidine-tagged wild type RfA2 from *Doryteuthis (Loligo) pealeii* (Genbank: ACZ57765.1) was synthesized and cloned into the pJExpress414 vector (DNA2.0). The vector was transformed into BL21(DE3) cells (Novagen). RfA2 was expressed at 37 °C using Overnight Express Instant Terrific Broth (TB) media (Novagen) supplemented with 100 μg mL $^{-1}$ Carbenicillin. RfA2 was insoluble when expressed at 37 °C and was sequestered in inclusion bodies. The inclusion bodies were then extracted by using BugBuster® (Novagen) according to the manufacturer's suggested protocols. The inclusion bodies were subsequently solubilized in denaturing buffer (pH 7.4, 50 mM sodium phosphate, 300 mM sodium chloride, 6 M guanidine hydrochloride) through repeated manual agitation and sequentially filtered through 5, 0.45, and 0.22 μm filters. The protein was next purified by high-performance liquid chromatography (HPLC) on an Agilent 1260 Infinity system using an Agilent reverse phase C18 column with a gradient evolved from 95% Buffer A:5% Buffer B to 5% Buffer A:95% Buffer B at a flow rate of 1 mL min^{-1} over 30 minutes (Buffer A: 99.9% H_2O, 0.1% TFA; Buffer B: 95% acetonitrile, 4.9% H_2O, 0.1% TFA) (Supporting Figure S1). The fractions containing RfA2 were pooled, flash frozen in liquid nitrogen, and

lyophilized, yielding > 200 mg of pure RfA2 protein per liter of *E. coli* cell culture.

(II) Characterization of Reflectin A2. Wild type RfA2 was characterized according to a general protocol, which was adopted from the literature[S1-S4]. Throughout the purification process, purified and unpurified reflectin samples were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and GelCode Blue Staining (Thermo) using an Invitrogen XCell *SureLock* Mini using NuPAGE Novex 4-12% Bis-Tris gels, with NuPAGE MOPS as the running buffer under reducing conditions. Stained protein bands were routinely subjected to in-gel tryptic digestion, as confirmation of protein identity[S1-S3]. After digestion, the peptides were separated on a reverse phase C18 chromatography column and analyzed by mass spectrometry either on a Synapt G2 instrument (Waters) outfitted with an electrospray ionization source or on an EASY-nLC system (Proxeon Biosystems, now Thermo Scientific) connected to a hybrid LTQ-FT spectrometer (Thermo Scientific) equipped with a nanoelectrospray ion source (Proxeon Biosystems, now Thermo Scientific)[S1,S4]. The resulting sequence coverages routinely exceeded > 80 % for RfA2 (Supporting Figure S2).

(III) Fabrication of Reflectin A2-based Devices. The two-terminal devices were fabricated using a protocol modified from established procedures[S2]. In brief, silicon dioxide/silicon or glass substrates (International Wafer Service, Inc.) were first cleaned in Piranha solution (1:3 hydrogen peroxide to sulfuric acid) and washed thoroughly. To fabricate devices for

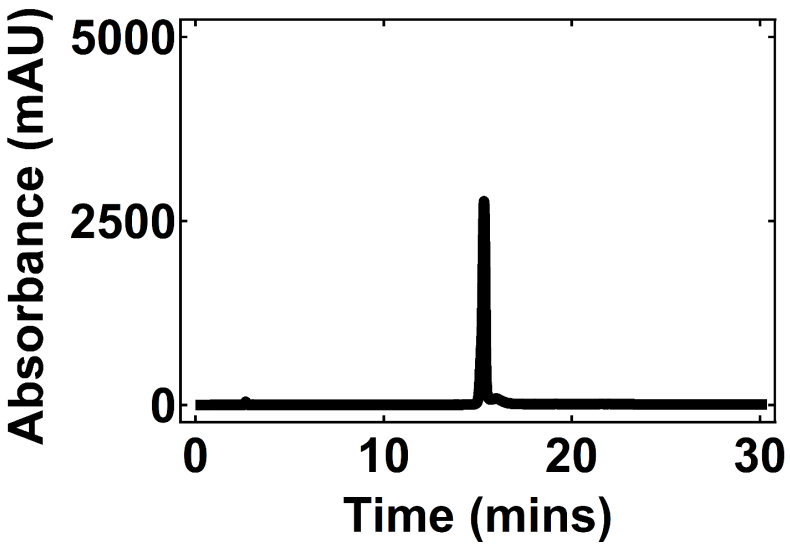
two-terminal direct current measurements, arrays of paired electrodes consisting of a 4 nm chromium adhesion layer overlaid with a 40 nm palladium layer were electron-beam evaporated onto SiO_2/Si substrates through a shadow mask. The dimensions of the palladium paired electrodes were 100 μm wide by 400 μm long, with an inter-electrode separation of 50 μm. To fabricate devices for two-terminal alternating current measurements, arrays of paired electrodes consisting of a 4 nm chromium adhesion layer overlaid with a 40 nm gold layer were electron-beam evaporated onto glass substrates through a shadow mask. The dimensions of the gold paired electrodes were 2.5 cm wide by 3 cm long with an inter-electrode separation of 100 μm. For all devices, aqueous solutions containing HPLC-purified RfA2 were prepared and subsequently dropcast onto the electrodes. The resulting films were dried in ambient conditions, and the excess material was scribed away mechanically, leaving the desired completed devices. To convert electron-injecting palladium (Pd) electrodes to proton-injecting palladium hydride (PdH_x) electrodes, the devices were exposed to a 5% hydrogen/95% argon atmosphere both before and during the electrical measurements.

(IV) Physical Characterization of Reflectin A2-based Devices. The devices were characterized with optical and atomic force microscopy, as previously described[S1,S2]. The dimensions of the reflectin films were determined from analysis of optical images obtained with a Zeiss Axio Imager A1 Microscope. The thicknesses of both dry and humidified reflectin films were determined from the analysis of topographical scans obtained with an

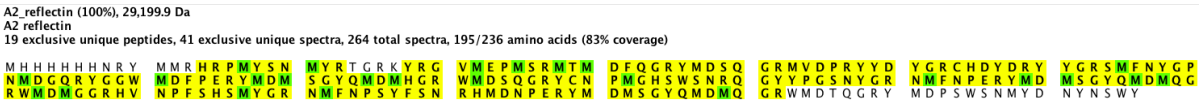
Asylum Research MFP-3D Atomic Force microscope outfitted with an Asylum Research Humidity Sensing Cell.

(V) Electrical Characterization of Reflectin A2-based Devices. The completed devices were characterized electrically in two different configurations according to established procedures[S2]. The direct current measurements were performed on a Cascade Microtech PM-5 Probe Station outfitted with an Agilent 4156C Semiconductor Parameter Analyzer, with the current was recorded as a function of voltage at a scan rate of ~ 0.6 V/s. The direct current measurements for palladium-contacted and palladium hydride-contacted devices were performed under 100% argon and 5% hydrogen/95% argon atmospheres, respectively. The alternating current measurements were performed with a 4294A Impedance Analyzer (Agilent) at various frequencies with a constant applied voltage of 500 mV. The alternating current measurements for gold-contacted devices were performed under a 100% argon atmospheres. All electrical experiments were performed at an 80% relative humidity, which was monitored with a Fisher Scientific hygrometer.

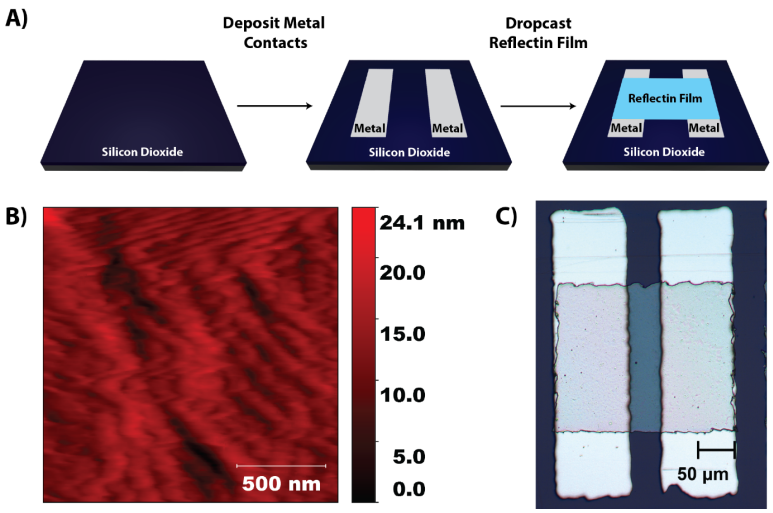
Supporting Figures:



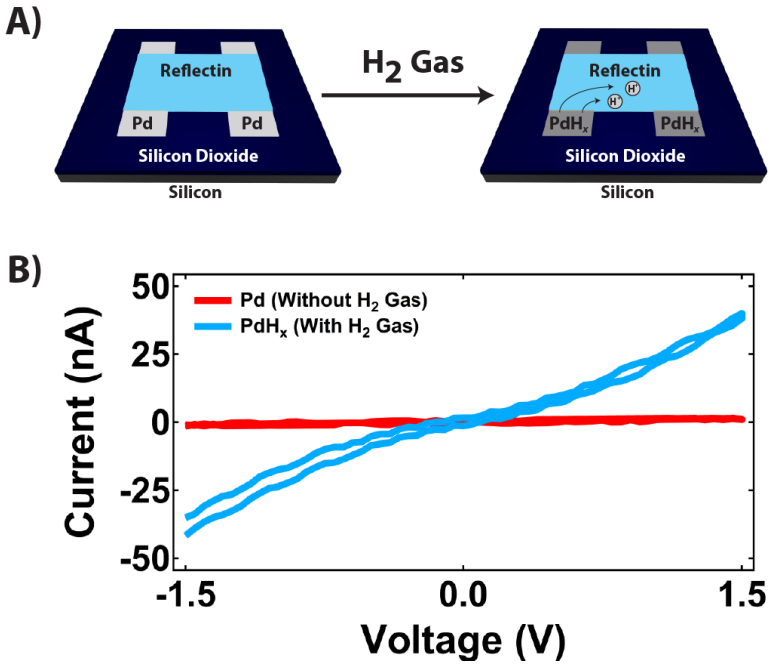
Supporting Figure S1: A typical analytical reverse phase HPLC chromatogram for RfA2 obtained after inclusion body filtration and concentration. The elution of the protein was monitored at a wavelength of 280 nm. The peak indicates excellent purity.



Supporting Figure S2: A tryptic peptide sequence coverage map obtained from tandem mass spectrometry analysis of the trypsin-digested of the histidine-tagged RfA2 protein. Bolded amino acids with a yellow background correspond to amino acids comprising tryptic peptides. Bolded amino acids with a green background correspond to oxidized amino acids comprising tryptic peptides. The total sequence coverage of ~ 83 % confirmed the purified protein's identity as RfA2 from *D. pealeii*.



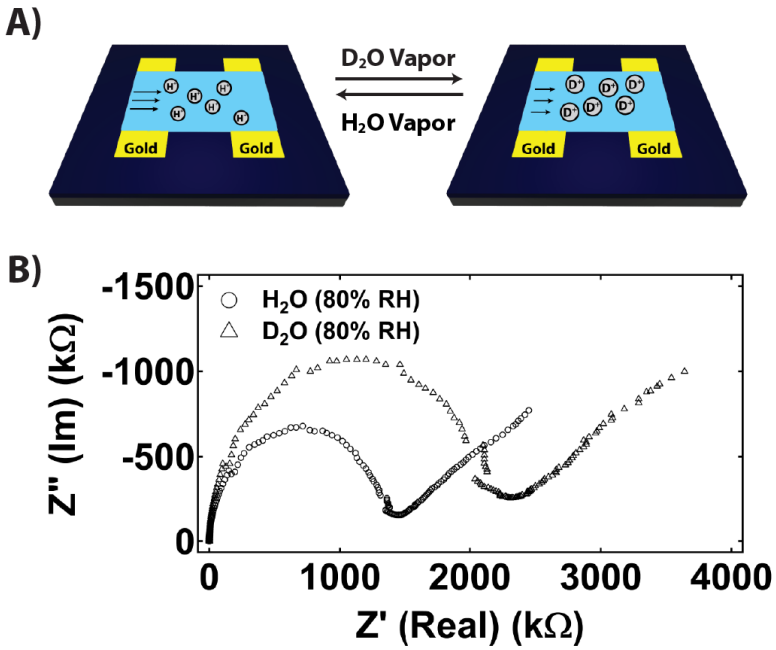
Supporting Figure S3: (A) General scheme for the fabrication of RfA2-based devices. (B) A representative optical image of a completed device for which an RfA2 film bridges two palladium electrodes. (C) A representative AFM image of an RfA2 active layer. The additional typical images correspond to Figure 2 in the main text.



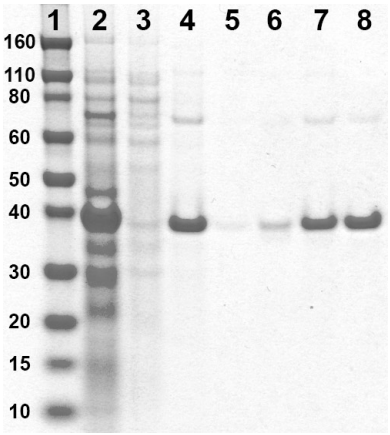
Supporting Figure S4: (A) An illustration of an RfA2-based two-terminal device before and after *in situ* treatment with hydrogen (H_2) gas. The palladium (Pd) electrodes are converted to palladium hydride (PdH_x) electrodes, enabling the injection of protons into the film. (B) The current versus voltage characteristics of an RfA2 film contacted with palladium (red) and palladium hydride (blue) electrodes. The magnitude of the current increases by more than an order of magnitude upon moving from proton-blocking to proton-injecting electrodes. Both the forward and reverse scans are shown for each measurement. The additional typical images correspond to Figure 3 in the main text.

```
A2              -MHHHHHHNRYMMRHRPMYSNMYRTGRKYRGVMEPMSRMTMDFQGRYMDSQGRMVDPRYY
A1              MAHHHHHHNRYLNRQR-LY-NMYRN--KYRGVMEPMSRMTMDFQGRYMDSQGRMVDPRYY

A2              D-YGRCHDYDRYYGRSMFNYGPNMDGQRYGGWMDFPERYMDMSGYQMDMHGRWMDSQGRY
A1              DYYGRMHDHDRYYGRSMFNQGHSMDSQRYGGWMDNPERYMDMSGYQMDMQGRWMDAQGRF

A2              CNPMGHSWSNRQGYYPG-----SNYGRNMFN-------------PERYMDMSGYQMDMQG
A1              NNPFGQMWHGRQGHYPGYMSSHSMYGRNMYNPYHSHYASRHFDSPERWMDMSGYQMDMQG

A2              RWMDMGGRHVNPFSHSMYGRNMFNP--SYFSNRHMDNPERYMDMSGYQMDMQGRWMDTQG
A1              RWMDNYGRYVNPFNHHMYGRNMCYPYGNHYYNRHMEHPERYMDMSGYQMDMQGRWMDTHG

A2              RYMDP---------------------------------------SWSNMYDNY-------
A1              RHCNPFGQMWHNRHGYYPGHPHGRNMFQPERWMDMSGYQMDMQGRWMDNYGRYVNPFSHN

A2              ------------------------------------NSW--------------------
A1              YGRHMNYPGGHYNYHHGRYMNHPERHMDMSSYQMDMHGRWMDNQGRYIDNFDRNYYDYHM

A2              Y
A1              Y
```

Supporting Figure S5: A sequence alignment of histidine-tagged reflectin A2 and histidine-tagged reflectin A1[S1,S2] from *D. pealeii.* The alignment was generated by using the MUSCLE software[S5,S6]. The charged amino acids (D, E, H, K, R) are highlighted in yellow, and they constitute 27.5% of the sequence for RfA2 and 30.3% of the sequence RfA1.



Supporting Figure S6. (A) An illustration of a two-terminal RfA2-based device in the presence of water vapor (H_2O) and deuterium oxide vapor (D_2O). (B) A representative Nyquist plot of the imaginary versus the real parts of the impedance for an RfA2-based two-terminal device in the presence of water (open circles) and deuterium oxide (open triangles). There is a change in the effective film resistance upon moving from H_2O to D_2O, illustrating the kinetic isotope effect for the RfA2 film. The additional typical images correspond to Figure 4 in the main text.



Supporting Figure S7. Analysis of the expression and purification of histidine-tagged RfA1[S1,S2] *via* sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The overall procedure was identical to the one described for RfA2 above. The stain indicates the total protein. The individual lanes correspond to: lane 1, 10-160 kDa molecular weight standards; lane 2, total protein; lane 3, soluble protein; lane 4, insoluble protein; lane 5, solubilized inclusion bodies; lane 6, filtered inclusion bodies; lane 7, concentrated filtered inclusion bodies; lane 8, HPLC eluate. After HPLC purification, RfA1 produced via this method was indistinguishable from the one produced via the previously reported protocols[S1,S2].

Supporting References:

(S1) L. Phan, W. G. Walkup IV, D. D. Ordinario, E. Karshalev, J.-M. Jocson, A. M. Burke and A. A. Gorodetsky, *Adv. Mater.,* 2013, **25**, 5621 – 5625.

(S2) D. D. Ordinario, L. Phan, W. G. Walkup IV, J.-M. Jocson, E. Karshalev, N. Hüsken, and A. A. Gorodetsky, *Nat. Chem.*, 2014, **6**, 596 – 602.

(S3) A. Kalli, and S. Hess, *Proteomics*, 2012, **12**, 21 – 31.

(S4) A. Shevchenko, H. Tomas, J. Havlis, J. V. Olsen, and M. Mann, *Nat. Protoc.,* 2006, **1**, 2856 – 2860.

(S5) R. C. Edgar, *Nucleic Acids Res.,* 2004, **32**, 1792 – 1797.

(S6) R.C. Edgar, *BMC Bioinf.*, 2004, **5**, 113.