Materials and Methods Supplement

Deposition of pyrite nanocrystal films on shape-memory polymer

All chemicals were used as received. Anhydrous iron (II) chloride (99.9% metal basis), sulfur powder (99.998%), anhydrous chloroform (≥99%), and anhydrous ethanol (99.5%) were purchased from Sigma Aldrich. Octadecylamine (90%) and diphenyl ether (99%) were purchased from Acros. All synthesis reactions were performed on a Schlenk line under air-free conditions. Briefly, a solution of 480 mg of sulfur in 10 mL of diphenyl ether was added to a solution of 368 mg of anhydrous $FeCl_2$ in 25 g of octadecylamine. Both precursor solutions were degassed at 75-85°C for 1 hr and then placed under flowing Ar atmosphere. After heating the octadecylamine solution to 218 °C, the ether solution was injected at 210 °C and the reaction stirred at 218 °C for 3 hr to grow the nanocrystals. The reaction was then quenched with a water bath. Once the solution temperature reached 95 °C, 20 mL of ethanol was injected to prevent the octadecylamine from solidifying. The quenched solution was immediately centrifuged at 4,400 rpm for 3 min and the resulting precipitate dispersed in 30 mL of chloroform. The nanocrystals were purified by two additional rounds of precipitation in ethanol and redispersion in chloroform. A final centrifugation at 3,000 rpm for 2 min was performed to remove any aggregates from the nanocrystal suspension. The resulting colloids (6 - 8 mg/mL) are stable for up to 2 wks when stored in air.

Time frame for pyrite shrink-wrap fabrication

The synthesis of the pyrite nanocrystals and the fabrication of pyrite shrink-wrap laminate take surprisingly little time. A total of eight and a half hours is required to fabricate eight 16-well chips (a total of 128 sample wells). The bulk of the time (7’ 45”) is for synthesis of the pyrite nanocrystals. Deposition of the nanocrystals, shrinking the coated polyolefin and thermoforming the sample wells requires an additional 45”. It should be noted that

fabrication of pyrite shrink-wrap laminate is amenable to scale up as all of the processes can be integrated into a roll-to-roll manufacturing line that would dramatically reduce the fabrication time per piece of pyrite shrink-wrap laminate.

Protein preparation and characterization

Aliquots of recombinant BirA tagged mouse Programmed Death 1 (PD-1) stored in 20 mM Tris‐HCl, pH 8.0 + 150 mM NaCl were thawed and dialyzed into standard reaction buffer (20 mM Sodium Cacodylate pH 8.0, 50 mM NaCl, 1 mM EDTA) overnight at 4 °C. Intrinsic fluorescence and sedimentation velocity analyses were performed to confirm that the protein retains its native fold. The intrinsic fluorescence of PD-1 was measured in a 3 mm quartz cuvette in a Fluoromax 3 spectrofluorometer in reaction buffer. Reference spectra for the denatured protein were obtained in this buffer to which 2 M guanidine was added.[1] Sedimentation velocity analysis of PD-1 was conducted in a Beckman XL-I analytical ultracentrifuge in the Ti-60 rotor at 58,000 rpm and 20 °C using the absorption optics set to 280 nm. Values of the sedimentation and diffusion coefficients were calculated using the time-derivative method implemented in the program DCDT+[2, 3] and corrected to standard conditions.[4] The fluorescence and sedimentation properties of PD-1 were assayed before and after the protein was incubated within microwells drawn into either bare shrunk polyolefin or laminate (without H_2O_2 or ascorbate present).

Measurement of oxidation by fluorescent dye degradation

Measurement of the fluorescence loss of an aromatic dye in solution is a convenient way to assess relative rates of •OH production. This assay was conducted following published protocols.[5-7] A stock solution of fluorescein (Molecular Probes, F1300) in standard reaction buffer was diluted such that the fluorescence of the final solution to be analyzed was within

the linear dynamic range of a Spectramax M5 plate reader (490 nm excitation, 520 nm emission and 515 nm emission cutoff).

MALDI-TOF mass spectral analysis of protein oxidation

One and one half μL of trypsin (sequence grade, Promega) was added to samples of either oxidized or unoxidized samples of PD-1 in the standard reaction buffer plus the quench solution that had been reduced using 98.5 μL of 0.02% proteaseMAX (Promega), 5 mM TCEP (Sigma), 20 mM iodoacetamide (Sigma) in 50 mM ammonium bicarbonate. A 1:20 ratio of trypsin to protein was added and digestion incubated for 3 hr at 37 °C with shaking. Decreasing the pH below 4 with 10% trifluoroacetic acid (TFA) terminates the reaction. Each digest was divided into aliquots, snap frozen on dry ice, and stored at -20°C. Individual volumes of each sample were concentrated and de-salted using C18 Zip-tips (Millipore). C18 tips were prepared using 100%, 50%, and 0% acetonitrile in the presence of 0.1% TFA. The sample aliquots were drawn into the resin to bind the peptides, followed by washing of the resin with 0.1% trifluoroacetic acid to remove salt, and finally eluted onto a MALDI plate using α-cyano-4-cinnamic acid in 70% acetonitrile/0.1% TFA.

Measurement of iron release from pyrite shrink-wrap laminate

To measure Fe^{2+} release from pyrite shrink-wrap laminate, 3 μL drops of solution containing 1 mM ascorbate and H_2O_2 at the indicated concentration are pipetted into wells and incubated with vibration for 60 s. The sample is collected and stock solutions of sodium acetate, hydroxylamine-HCl and 1,10'-phenanthroline are added to final concentrations of 120, 6 and 500 mM, respectively. The final volume of 1.2 mL is incubated for 10 min at room temperature. The 508 nm absorption of the Fe^{2+} - 1,10'-phenanthroline chelate is compared to

a calibration curve constructed from known concentrations of ferrous ammonium sulfate hexahydrate (Sigma).[8]

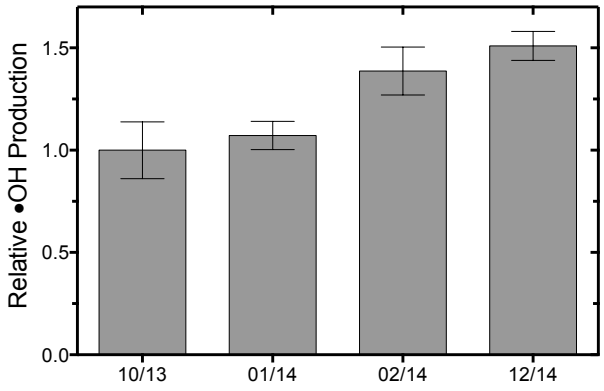
Hydroxyl radical generation using $[Fe(edta)]^{2-}$

Footprinting using $[Fe(edta)]^{2-}$ was conducted as described in [7, 9]. Briefly, dye (see *Supplement*) is diluted to the desired concentration in the standard reaction buffer and 30 µL is aliquoted into a microfuge tube. Drops of 0.6 µL of fresh stock solutions of ascorbate, H_2O_2, and EDTA-$(NH_4)_2Fe(SO_4)_2{\cdot}6H_2O$ are added to final concentrations of 10 mM, 15 mM and 10 µM, respectively. Vortexing initiates oxidation, which is quenched after 1 min as described in the main article for pyrite shrink-wrap laminate.

Results Supplement

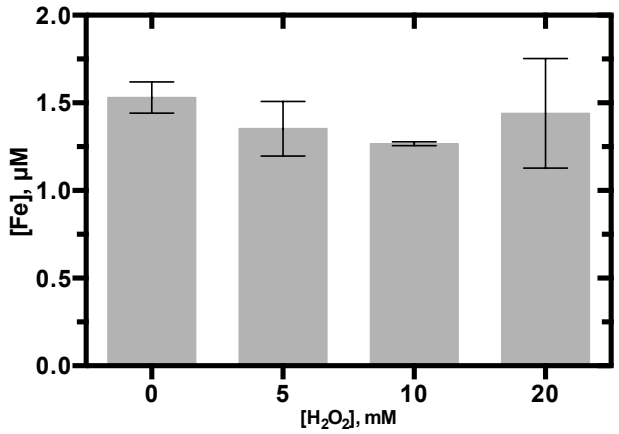
Supplementary Video 1: See enclosed file 'PSWL - Pyrite Deposition Video.m4v'

Supplementary Figure 1: Pyrite shrink-wrap laminate stores stably for at least one year



The •OH production from pyrite shrink-wrap laminate quantified as a function of storage time in a closed container at room temperature, in the dark and ambient humidity using the dye degradation assay described above at constant experimental conditions. A 3 µL drop containing dye, 8 mM H_2O_2 and 1 mM ascorbate was incubated with vibration for 60 sec and quenched following our standard protocol. The relative •OH production was normalized to the first measurement taken in October of 2013. Pyrite shrink-wrap laminate clearly does not lose activity when stored over the 14 months assayed. It is not clear why the measured relative activity increases subsequent to our initial measurement. A plausible rationalization is that subtle improvements in the conduct of experiments yield this apparent improvement.

Supplementary Figure 2: Ferrous iron release from pyrite shrink-wrap laminate

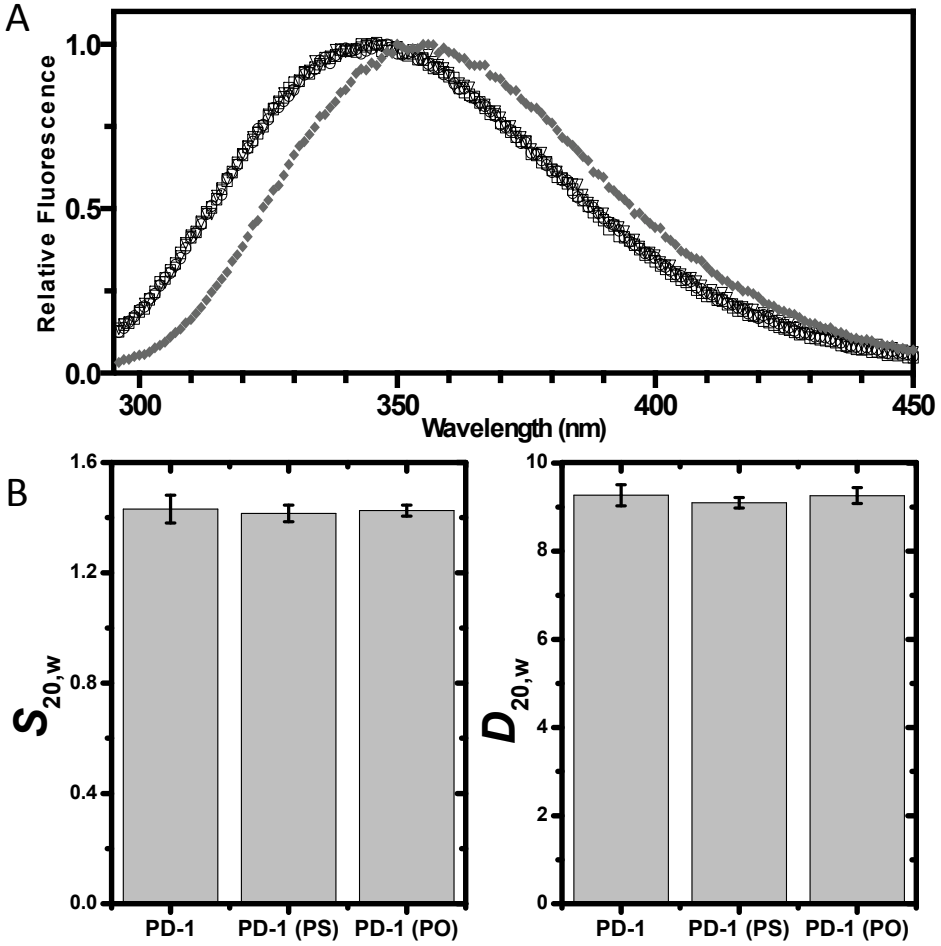


Measurement of the amount of iron released from pyrite shrink-wrap laminate during production of •OH. Three µL buffered drops containing 1 mM ascorbate, and the indicated concentration of H_2O_2 was incubated on pyrite shrink-wrap for 1 min with vibration. The released ferrous iron was assayed by its chelation with 1,10'-phenanthroline as described above in this *Supplement*.

Supplementary Figure 3: The native fold of PD-1 is unaffected by exposure to either bare shrink-wrap or pyrite shrink-wrap laminate

Prior to demonstrating protein oxidation, control experiments were conducted to the PD-1 protein retains its native conformation following exposure to bare shrink plastic and pyrite shrink-wrap laminate surface (*Supplementary* Fig. 1). We verified that the protein is monomeric under our solution conditions by sedimentation equilibrium analysis (M_w = 14,967 ± 627 Da). Analytical sedimentation velocity reports the global conformation of a monodisperse protein; the sedimentation profiles of a control PD-1 sample and PD-1 incubated on bare shrink plastic or pyrite-shrink are indistinguishable; denatured protein would display slower sedimentation and diffusion rates. Intrinsic tryptophan fluorescence is an established measure of the stability of protein folds. When tryptophan residues move from a hydrophobic protein core toward solution, the emission maximum of tryptophan shifts to longer wavelengths. The emission spectra of PD-1 incubated on either material are indistinguishable from the control spectra that are readily distinguished from the spectra of

the denatured protein. Two independent measures confirm that neither the plastic substrate nor pyrite shrink-wrap laminate surface denature the PD-1 protein.



Contact with pyrite shrink laminate does not cause denaturation of PD-1. Drops containing PD-1 in standard reaction buffer were incubated on the surfaces for 25 min with vibration prior to analysis of the protein's global conformation. **(A)** Intrinsic tryptophan fluorescence was used to determine whether exposure of the protein PD-1 (□) to bare polyolefin (O) or pyrite shrink laminate (Δ) unfolds the protein. PD-1 denatured in 2 M urea (shaded ◆) is shown for comparison. **(B)** Sedimentation velocity analysis measures the global conformation of biological macromolecules. The native conformation of PD-1 is maintained following exposure of the protein to bare polyolefin or pyrite shrink laminate is the invariance of the measured sedimentation (S) and diffusion coefficients (D). S and D are corrected to $S_{20,w}$ and $D_{20,w}$ to account for temperature (20°C) and buffer properties within the experiment.

Literature Cited

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