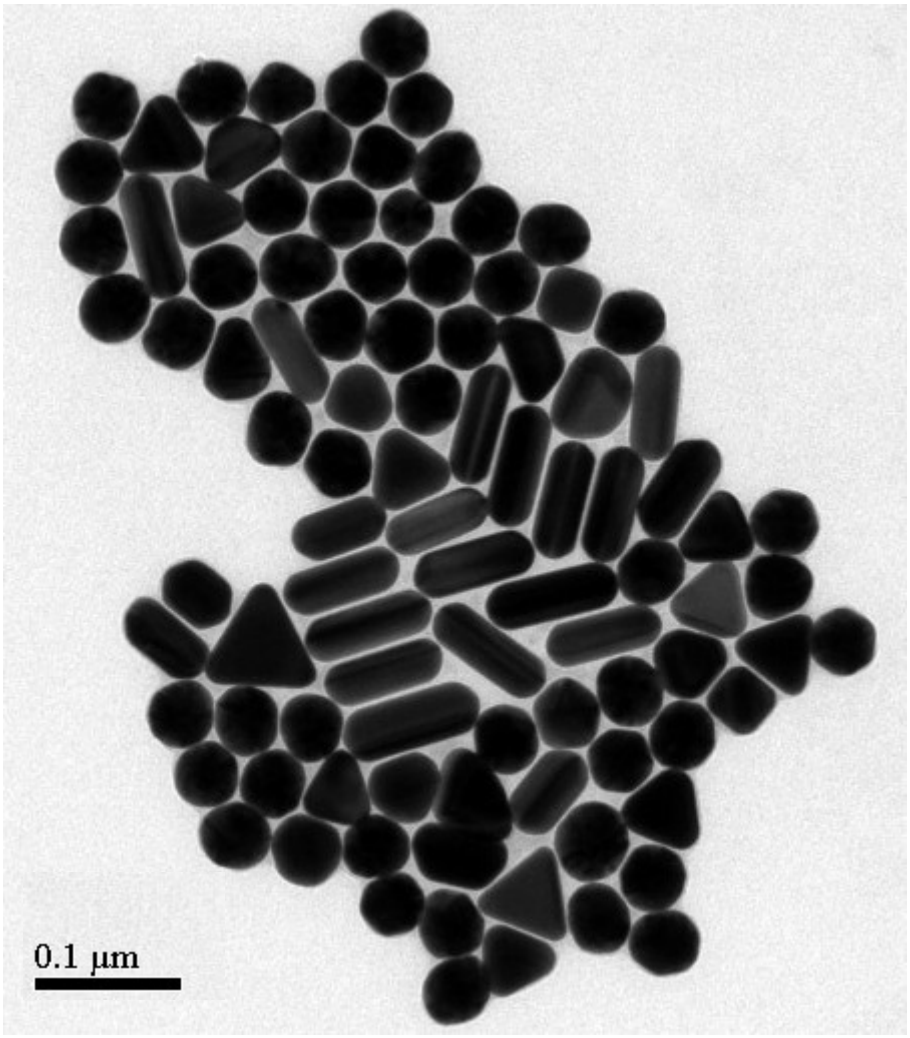
Gold nanorods were synthesized and functionalized as described previously[12]. The F_1-ATPase used in a typical experiment was isolated from *E. coli* and modified to contain a 6Xhis tag on the α-subunit N-terminus, and a γ-subunit cysteine substitution (γS193C) covalently modified with biotin-maleimide[12]. The ATPase activity of the enzyme had a k_{cat} = 130 sec^{-1} as measured by a coupled assay in bulk solution[12]. In single molecule rotation experiments, we have observed up to 65% of the F_1 molecules rotate [24]. The biotinylated F_1-ATPase was immobilized on a Ni-NTA column to which saturating amounts of avidin were added to ensure that all of the biotin groups were bound to avidin. After an F_1-ATPase buffer (50 mM Tris, pH 8.0, 10 mM KCl) wash to remove excess avidin, avidinated F_1-ATPase was eluted from the column and 10 μl of 50 μg/ml of avidinated F_1-ATPase was incubated on a Ni-NTA coated cover slip for 5 min. The surface was rinsed with buffer for 30 sec, then wicked to remove excess liquid.

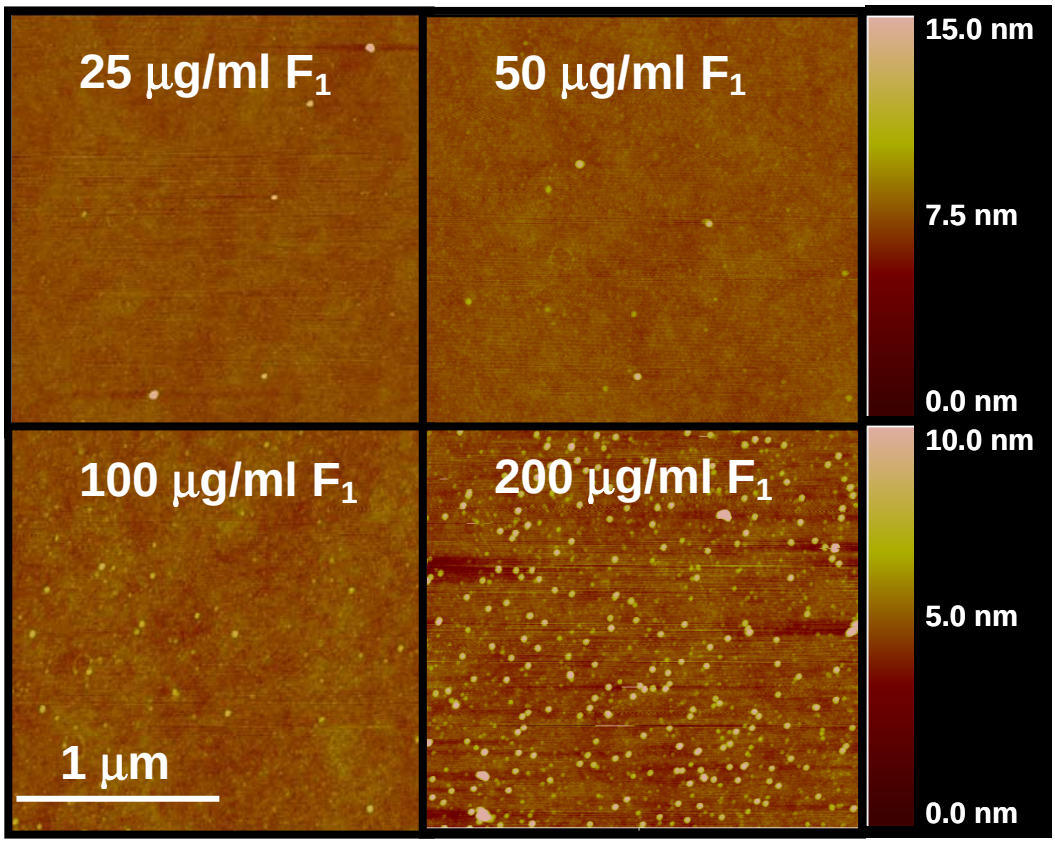


Supplementary Figure 1: Transmission Electron Micrograph of Gold Nanoparticles used for detection.

The supplemental movie shows F_1-ATPase dependent rotation of gold nanorods bound via DNA bridges. In this example, 600 molecules of DNA were added to the coverslip. The movie covers about ~15% of a full field of view. Two of the five gold nanorods show significant rotational activity, while 2 others show minor fluctuations in intensity. The movie was collected at 53 fps, which is not fast enough to capture all rotational events. However, this rate is fast enough to observe the strobe effect, which allows identification of rotating gold nanorods as shown previously (12).

For experiments in which LXR products prepared from target DNA were used for detection, target DNA, 3'-biotinylated,5'-phosphorylated capture probe, and 5'biotinylated capture probe were allowed to hybridize, and the capture probes were ligated in the presence of 400 units of T4 ligase, T4 ligase buffer (New England Biolabs), 4 mM Mg^{2+}ATP, and 4 mM DTT in a final volume of 50 μl overnight. After ligation, 35 μl of the product was incubated with 20 units of DNA polymerase-ϕ29, ϕ29 buffer (New England Biolabs), 2 mM dNTP, and 0.2 μg of BSA in a 50 μl total volume for 3 hours. All reactions were performed at room temperature with an excess of incubation time to ensure completion of ligation and exonucleation reactions during LXR. No attempts were made to optimize incubation times.

Atomic force microscopy (AFM) was used to determine an appropriate concentration of F_1-ATPase to ensure that: (1) two 3'5'-dibiotinylated DNA bridges could not crosslink to the same gold nanorod; (2) a single 3'5'-dibiotinylated DNA would not span between two F_1-ATPases; (3) two gold rods were unable to interfere with one another during rotation; and (4) individual rods were distinguishable as limited by the diffraction limit of light. Supplementary Figure 1 shows AFM images of the distribution of F_1 molecules on a surface at concentrations of 25, 50, 100, and 200 μg/ml. The average distance between F_1 molecules was 1± 0.5 μm at 50 μg/ml, sufficient to meet the above criteria (Supplemental Figure 1). Using this concentration, we estimate an average of 10,000 F_1 molecules to be bound in an area approximately equal to a field of view in the microscope (31400 μm^2).



Supplementary Figure 2: Atomic Force Microscopy of F_1 Bound to the Coverslip. F_1 molecules were added to a glass slide at concentrations of 25, 50, 100, and 200 μg/ml. Bound F_1 molecules are indicated as yellow spots, corresponding to a height of ~ 9 nm.