Supporting Information

A DNA-templated fluorescent silver nanocluster with enhanced stability

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DNA sequences:

J1' – 5'-CCCCCCCCCCCC-3' (ref. 1)
J6' – 5'-CCCTAACTCCCC-3' (ref. 2)
J7' – 5'-AATTCCCCCCCCCCCCCAATT-3' (ref. 2)
J12' – 5'-AGTCACCCCAACCTGCCCTACCACGGACT-3' (ref. 3)
J13'– 5'-GGCAGGTTGGGGTGACTAAAAACCCTTAATCCCC-3' (ref. 3)
J14'– 5'-AGTCCGTGGTAGGGCAGGTTGGGGTGACTAAAAACCCTTAATCCCC-3v (ref. 3)
J15'– 5'-TATCCGTCCCCCCCCCACGGATA-3' (ref. 4)
I-motif' 5'-CCCCTAACCCCTAACCCCTAACCCC-3' (ref. 5)
Th'– 5'-GGGGGGCCTGCCCTACCACAAAACCCTTAATCCCC-3'
D'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-D'– 5'-AACCGAACCTGGGCTACCACCCTTAATCCCC-3'
D1'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D2'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D3'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D4'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D5'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D6'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D7'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D8'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D9'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D10'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D11'– 5'-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Da'– 5'-CCCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Db'– 5'-GCCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Dc'– 5'-TCCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Dd'– 5'-AACCGAACCTGGGCTACCACCCTTAATCCCC-3'
De'– 5'-ATCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Df'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Dg'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Dh'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Di'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Dj'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Dk'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Dl'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Dm'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Dn'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-I'– 5'-CCCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-II'– 5'-GCCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-III'– 5'-TCCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-IV'– 5'-AACCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-V'– 5'-ATCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-VI'– 5'-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-A'– 5'-CCCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-B'– 5'-GCCCGAACCTGGGCTACCACCCTTAATCCCC-3'
D-C'– 5'-TCCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Ran1'– 5'-TTCGACCTGAGGCCAATCCACCCTCCACACC-3'
Ran2'– 5'-CAACGCAGCTGCCTCCTTACCACCCACCTAG-3'
Ran3'– 5'-CGGCACAACTACCTCCACCGCTTGCCTAACC-3'
Ran4'– 5'-CACACAGCACACACAGCTCTCTCTCTCGCGC-3'

(Bases in gray indicate those that have been deleted from the D'sequence. Bases in red are substitutions)

Section S1.

Synthesis of AgNCs: Briefly, 15 μM DNA (Integrated DNA Technologies) and 90 μM $AgNO_3$ (Sigma Aldrich) were sequentially added and mixed with sodium phosphate buffer (20 mM, pH 6.8), and the reaction mixture was incubated at room temperature, in the dark, for 20 minutes. Freshly prepared $NaBH_4$ (90 μM final concentration, Sigma Aldrich) was added and the reaction rapidly mixed and incubated at room temperature, in the dark, for 4 hours.

Circular Dichroism (CD): Circular Dichroism studies were performed using a Jasco J-710 Spectropolarimeter. All AgNCs were prepared employing the above synthetic procedure.

Determination of quantum yield: Quantum yields of D-AgNCs were determined using gradient method[6] employing Rhodamine 101 dissolved in methanol as a reference.[7]

Fluorescence measurements: All fluorescence measurements were made using a Varian Cary Eclipse fluorescence spectrophotometer. Integrated fluorescence was defined as overall emission intensity in the range of 100 nm surrounding the emission peak.

Thermal stability: Temperature stability was determined by scanning the fluorescence of the AgNC as a function of temperature using a Stratagene Mx3000P QPCR system. Sample tops were covered with 20 μL of mineral oil to avoid evaporation. Fluorescence was observed using a ROX filter (575 nm/ 602 nm) with two minute stops at each step of 5 °C (the total time taken from 25 °C to 95 °C was two hours).

Electrochemistry: Electrochemical experiments were performed using a Bioanalytical Systems model CV-50W potentiostat. Our three-electrode setup for cyclic voltammetry consisted of a glassy carbon working electrode (disk Ø = 3.0 mm), a Pt wire auxiliary electrode, and a standard Ag/AgCl reference electrode (E^{o} = +0.196 V vs NHE). Standard protocols were used as recommended for cleaning and treating the GC working electrode, which was always carefully examined by analyzing the voltammograms of the supporting electrolyte alone (blank) before sample measurements. Cyclic voltammograms were recorded at scan rates of 10–10,000 mV s^{-1} and let run for at least 3 full cycles. Although voltammograms were also obtained in the entire electrochemical window of water, no additional cathodic or anodic process were observed outside the selected potential range (–0.7 to +0.8 V vs Ag/AgCl). The 0.05 M phosphate buffer used in sample preparations was the only electrolyte source in all cases reported here. For bulk electrolysis, a two-compartment cell was used in order to separate the Pt counter electrode from the main vial containing the stirring sample under oxidation; electrochemical contact between compartments was made through a porous Vycor glass frit. All sample solutions were deoxygenated and then blanketed with an argon atmosphere throughout the experiments.

In vivo experiments: THP-1 monocytes (ATCC TIB 202) were cultured in RPMI-1640 medium (ATCC 30-2001) supplemented with 10% fetal calf serum (HyClone SH30109.02) and 1% penicillin/streptomycin (Gibco 15140). Cells were seeded at $2x10^5$ cells per well in a 24-well plate (Corning 3524). The monocytes were differentiated into macrophages by incubation in 20 nM *12*-0-tetradecanoylphorbal-*13*-acetate (PMA; Acros 356150010; diluted in RPMI) at 37 degrees C, with 100 % humidity and 5% $C0_2$ for 48 hours. The cells were then washed three times with warm PMA-free media and incubated for 4 hours before treatment with either AgNC or DNA alone or silver alone. These samples were all diluted in 20 mM phosphate buffer (no salt), pH 6.8. Cells were incubated with 5 μM DNA, 5 uM AgNC, and 5 μM or 50 μM silver nitrate for 60 minutes. A sample of PMA-treated, washed cells incubated with only the same volume of 20 mM PB for 60 minutes was also included. The cells were counted for intactness (health) or the cells were then scraped from the wells and treated with trypan blue exclusion staining for live/dead analysis. Upon visual inspection with optical microscopy, healthy cells show a characteristic morphology, which includes a definite, distinct cell membrane, which appears dark due to the fact that the healthy cells are fully spherical. As cells start to sicken and die, their cell membranes break down, and they become flattened as the cytoplasm leaks into the surrounding medium. As they flatten, they appear pale due to the change in refractive index, and their membranes appear granular instead of smooth.

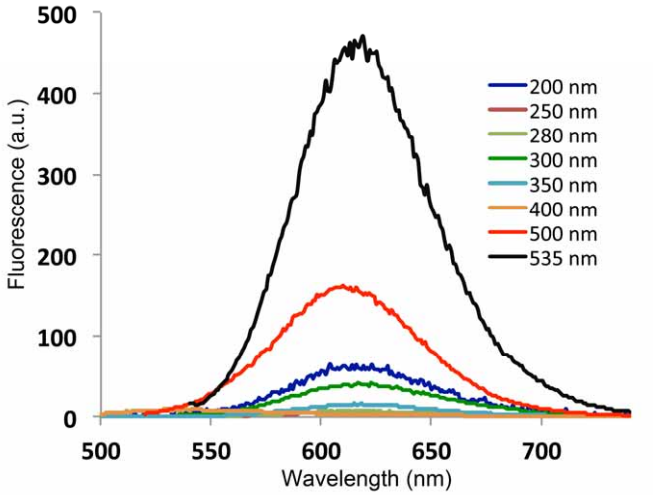


Figure S1. Fluorescence spectra of D-AgNC at different excitation wavelengths.

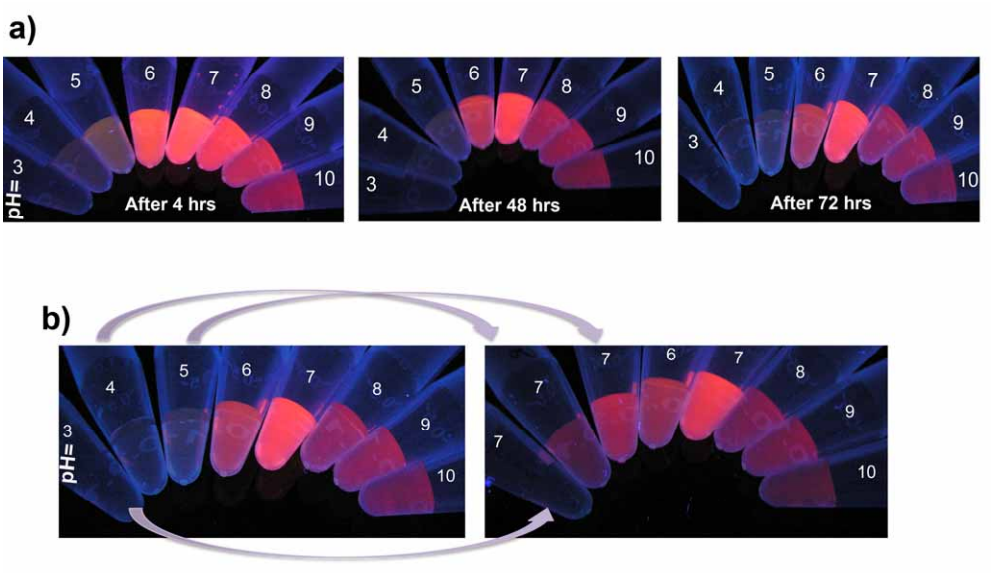
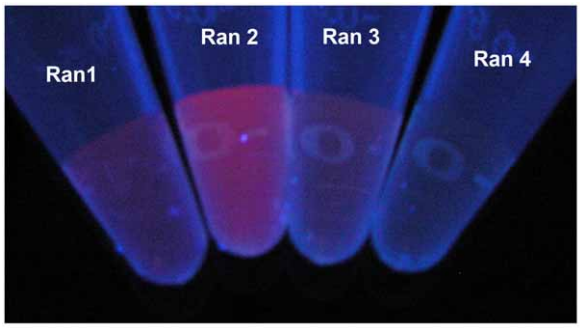
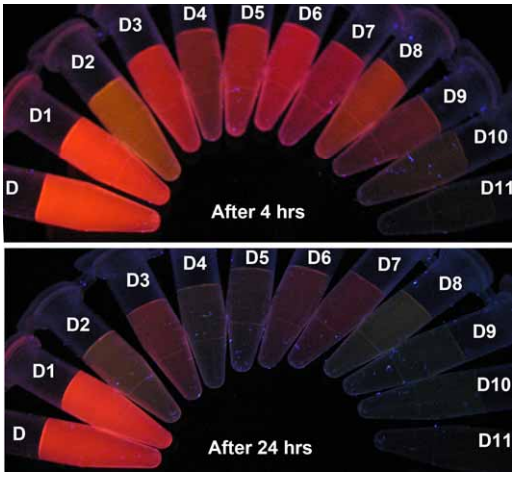


Figure S2. Stability of D-AgNC with change in pH. a) Effect of pH on stability and emission intensity of D-AgNC, b) Samples were synthesized at pH 3, 4, and 5, and after 72 hours the pH was raised to 7 and the fluorescence monitored.



D' - 5′ -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3'
Ran1' - 5′ -TTCGACCTGAGGCCAATCCACCCTCCACACC-3'
Ran2' - 5' -CAACGCAGCTGCCTCCTTACCACCCACCTAG-3'
Ran3' - 5′ -CGGCACAACTACCTCCACCGCTTGCCTAACC-3'
Ran4' - 5′ -CACACAGCACACACAGCTCTCTCTCTCGCGC-3'

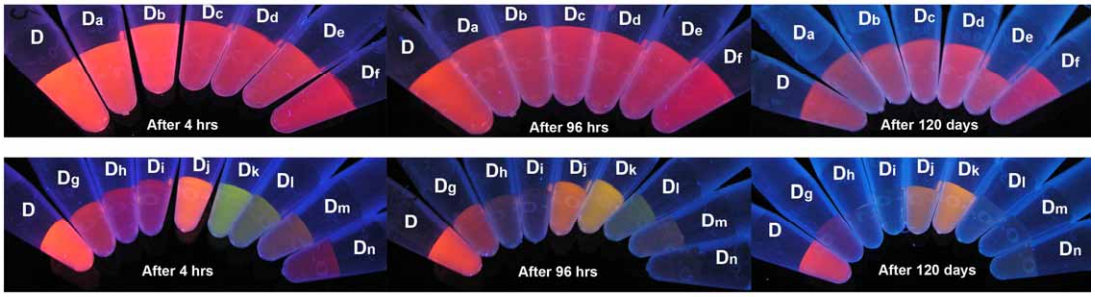
Figure S3. DNA sequences of D and randomized DNA strands. a) Photographs of AgNCs synthesized by randomizing the D' sequence and keeping the number of each type of base (A, G, C, and T) the same.



D' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D1' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D2' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D3' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D4' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D5' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D6' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D7' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D8' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D9' – **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D10'– **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D11'– **5′** -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′

Bases in gray are those that have been deleted from the D' sequence.

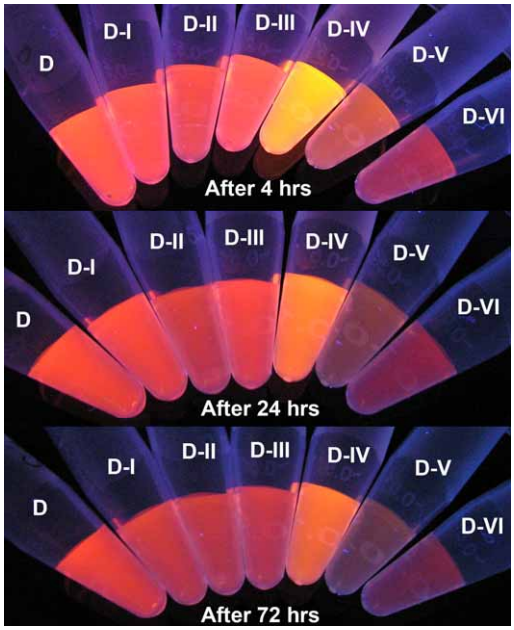
Figure S4. Effect on fluorescence stability and emission wavelength of AgNCs synthesized using step-by-step deletion of bases from the 5′- end of D'.



D' – 5′ -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Da' – 5′ -CCCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Db' – 5′ -GCCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Dc' – 5′ -TCCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Dd' – 5′ -AACCGAACCTGGGCTACCACCCTTAATCCCC-3′
De' – 5′ -ATCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Df' – 5′ -AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Dg' – 5′ -AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Dh' – 5′ -AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Di' – 5′ -AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Dj' – 5′ -AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Dk' – 5′ -AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Dl' – 5′ -AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Dm'– 5′ -AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′
Dn' – 5′ -AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′

Bases in gray are those that have been deleted from the D' sequence.

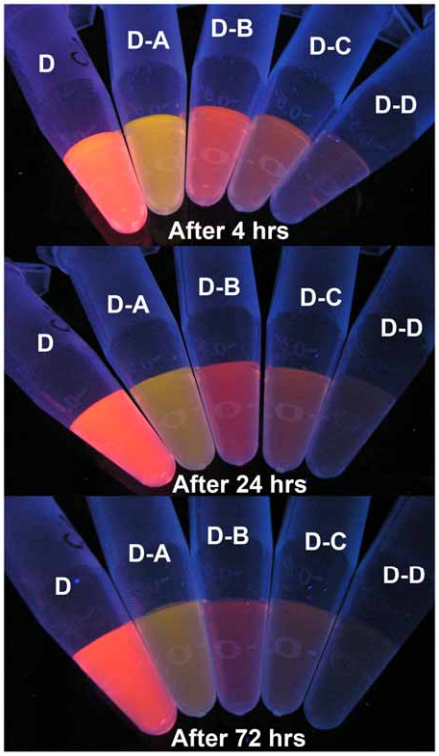
Figure S5. Effect on fluorescence stability and emission wavelength of AgNCs synthesized using step-by-step deletion of internal bases.



D'	**– 5′**	-ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-I'	**– 5′**	-CCCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-II'	**– 5′**	-GCCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-III'	**– 5′**	-TCCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-IV'	**– 5′**	-AACCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-V'	**– 5′**	-ATCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-VI'	**– 5′**	-AGCCGAACCTGGGCTACCACCCTTAATCCCC-3′

Bases in red are substitutions relative to D' sequence.

Figure S6. Effect on fluorescence stability and emission wavelength of AgNCs synthesized using step-by-step replacement of 5′- adenine and cytosine of sequence D'.



D' – 5′- -ACCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-A' – 5′ -CCCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-B'– 5′ -GCCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-C' – 5′ -TCCCGAACCTGGGCTACCACCCTTAATCCCC-3′
D-D' – 5′ -AACCGAACCTGGGCTACCACCCTTAATCCCC-3′

Bases in gray are those that have been deleted from the D' sequence.

Figure S7. Effect on fluorescence stability and emission wavelength of AgNCs synthesized using random deletion of internal bases.

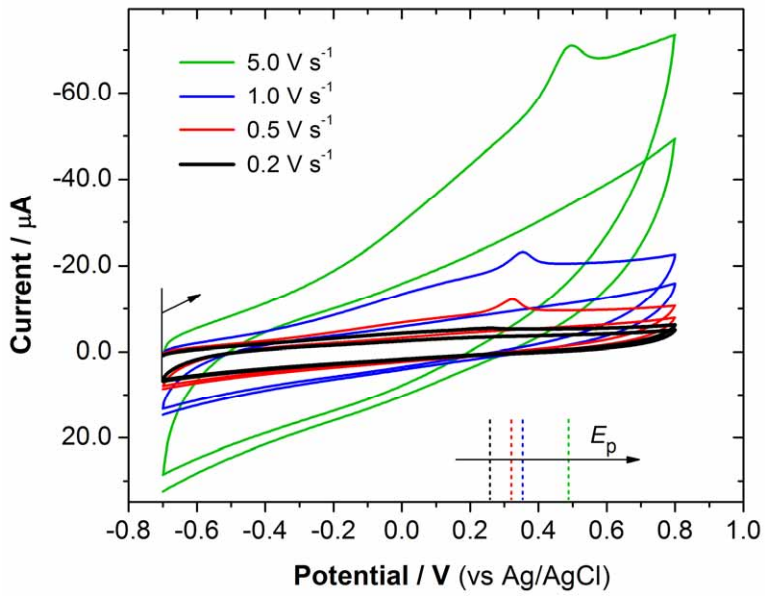


Figure S8. Cyclic voltammograms of D-AgNC (50 mM PB solution; pH 6.8) obtained at selected potential scan rates (v). The variation of peak potentials (E_p) with v is illustrated in the figure.

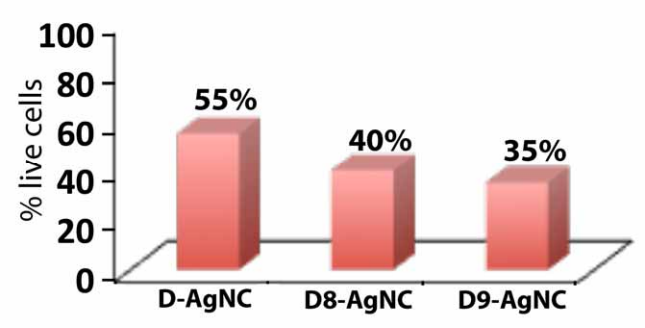


Figure S9. Percent live cells after addition of 5 □M AgNC to macrophages. Live/dead trends tend to correlate to observational health (Fig. 6).

References:

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