Supporting Information

Red-emitting p53-protected Gold Nanoclusters and Their Screening on Anti-tumor Agents from Chinese Medicine

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Fig. S1 The SDS-PAGE of the recombinant p53 core protein (22 kDa) expressed from E. coli.



Fig. S2 The fluorescence spectra to show the optimization of A) concentration of p53 at 5, 10, 15, 20 mg/ml; and B) reaction temperature at 37, 55, 70, 80 °C, respectively.



Fig. S3 The comparison of fluorescence spectrum for p53-AuNCs (black line) and that of p53 and NaOH (red line) after heated for 24 h at the identical experimental conditions. After 24 h, the red line showed no red-emission without the participation of HAuCl₄.



Fig. S4 A) The fluorescence spectra of the p53-AuNCs in aqueous solution (0.01 mg/ml) in the presence of different concentrations of NaCl (0 - 1.0 M); and B) the corresponding histogram.



Fig. S5 The luminescence response of p53-AuNCs (0.01 mg/ml) to A) different pH in PBS, p53-AuNCs stay stably at the alkaline conditions but the luminescence is quenched a little bit at acid environment although it is strongest at neutral one (pH 7.0); B) different buffer solution of PBS, Tris-HCl, and HEPES, respectively, at pH 7.4, the intensity are pretty stable at different kinds of buffer solutions.



Fig. S6 A) The TEM image of p53-AuNCs particles being used for the statistics of particle size; B) the corresponding energy dispersive spectrum (EDS), where the element of Au can be observed clearly.



Fig. S7 A) Fluorescence spectra of several flavonals in water (50 μ M) with 0.1% DMSO, respectively; B) The fluorescence spectra of p53-AuNCs (0.01 mg/ml) in the absence and presence of myricetin, morin, hypericin, and apigenin (50 μ M) in water with 0.1% DMSO, respectively; all the spectra were measured at room temperature with an incubation of 80 min after mixing ($\lambda_{ex} = 420$ nm).



Fig. S8 Time-dependent (A) fluorescence spectra and (B) the corresponding intensity at 676 nm of p53-AuNCs (0.01 mg/ml) in mixing with myricetin (50 μ M) in water.



Fig. S9 UV-vis absorption spectrum of 1.0 mM p53, 20 μ M myricetin and that of 20 μ M myricetin in the presence of different concentrations of p53 (1.0, 1.5 mM). A new absorption peak at ~380 nm appears and red-shifts with more p53 addition (red, green and blue line), indicating myricetin is combined with p53 in forming a complex first and then lead the aggregation of AuNCs. Therefore, it provides direct evidence for such process.



Fig. S10 A) The TEM image of p53-AuNCs particles after adding myricetin; B) the corresponding energy dispersive spectrum (EDS), where the element of Au can be observed clearly, which provides direct evidence on the induced aggregation of AuNCs.



Fig. S11 Cell images for Hela in the presence of p53 (2 mg/ml) (top) and the blank control (bottom) with the scale bar of 20 μ m. Both are performed in the absence of p53-AuNCs to finally exclude the cells auto-fluorescence in Figure 5.



Fig. S12 (A) Cytotoxicity of p53-AuNCs (0.6 mg/ml) on the growth of *E. coli* basing on the optical density (OD) at 600 nm, where slight difference is observed between the test (red) and control (black) groups during 12 h; (B) Viability of HeLa cells being treated with varying concentration of p53-AuNCs (0-3.6 mg/ml) for 24 h by using the MTT assay.



Scheme S1 Schematic structure of Apigenin, Morin, Myricetin and Hypericin, respectively.

Sample	$ au_{l}(s)$	α_l	$ au_2(s)$	α_2	τ_a (s)
1 mM p53-AuNCs	6.262×10 ⁻⁷	16.64 %	3.136×10 ⁻⁶	83.36 %	3.040×10 ⁻⁶
1 mM p53-AuNCs	6.693×10 ⁻⁷	23.02 %	2.002×10-6	76.98 %	1.881×10-6
+ 50 µM myricetin					
1 mM p53-AuNCs	6.816×10 ⁻⁷	26.32 %	1.908×10-6	73.68 %	1.805×10 ⁻⁶
+ 100 µM myricetin					

Table S1 Fitting results using Global analysis for the decay curves of Figure 3B.

^a a_i represents the amplitudes of components *i* at t = 0; τ_i is the decay time of component *i*; τ_a is the average lifetime.