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Electronic Supplementary Information

<u>for:</u>

Enzymatic digestion of luminescent albumin-stabilized gold nanoclusters under anaerobic conditions: clues to the quenching mechanism.

by

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* Corresponding author: W. Dzwolak Phone: +48 22 552 6567 Fax: +48 22 552 4029 E-mail: <u>wdzwolak@chem.uw.edu.pl</u> SDA-PAGE analysis of partly digested BSA-AuNC illuminated with theemission-inducing 365 nm light.



Figure S1. SDA-PAGE of partly digested BSA-AuNC. Photos of the gel taken in daylight and in 365 nm LED light.

Luminescent BSA-AuNC was digested with proteinase K at the 100:1 mass ratio and under the typical conditions used in this study (pH 7.5, 37 °C) for various periods of time: 0 min (non-digested at all – lane I), 5 minutes (lane IV), 1 h (lane III), and 24 hours (lane II); lane V is for to the molecular weight markers. The samples (9.5 μ g portion of each) were transferred to polyacrylamide gel and subjected to electrophoresis, as described in the Materials and Methods section. However, right after the electrophoresis but prior to the staining step, a photo of the gel illuminated with strong monochromatic beam of 365 nm light from a LED source was taken (A). In order to remove halo from scattered UV light, the photo was taken through a dense red optical filter with a cut-off wavelength of approximately 530 nm (exposition time ~ 30 seconds). Subsequently, the staining protocol was completed and another photo was taken in daylight (B).

The outcome of this experiment indicates that proteinase K is very effective in partial fragmentation of luminescent BSA-AuNC which are initially strongly agglomerated (BSA molecules are possibly cross-linked through the binding to sandwiched AuNCs, as well as via other bonds and interactions formed during the synthesis of the conjugate). This fragmentation does not result in the immediate quenching of the luminescence, but produces smaller BSA-AuNC conjugate species capable of entering the polyacrylamide gel. These species are highly heterogeneous which is reflected by their appearance as long smears (250 \sim 25 kDa). Interestingly, the prolongation of digestion from 5 minutes to 1 hour has only a limited impact on the appearance of the smear which, predictably, shift to lower molecular weights range and only slightly loses its luminescent intensity. The complete quenching requires longer digestion times.