SUPPLEMENTARY MATERIAL

Gold nanoclusters as elemental label for the sequential quantification of apolipoprotein E and metallothionein 2A in individual human cells of the retinal pigment epithelium using single cell-ICP-MS

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DESCRIPTION OF THE SUPPLEMENTARY MATERIAL

This Electronic Supporting Material contains details about the Experimental section as well as some additional comments about Results and Discussion section.

The Experimental section describes the experimental seps to perform the synthesis of the metal immunoprobes used for the detection of both proteins. Additionally, a diagram of whole cell process is collected.

On the other hand, in the Results and Discussion section, results regarding immunoassay optimization as well as some comments about the APOE quantification is also collected.

EXPERIMENTAL METHODS

Synthesis of the AuNCs:anti-h-APOE and AuNCs:anti-h-MT2A immunoprobes

In order to synthesis the metal immunoprobes the carbodiimide strategy was followed. Thus, a molar ratio of 1:3:1200:1200 (antibody:AuNCs:EDC:NHS) was employed, by using 100 µl of each antibody with a concentration of 100 µg mL^{-1}. The labelling is finished after 2 h of vortex shaking at room temperature. Then, the purification using Amicon units (100 kDa cut-off) of the immunoprobes is required to remove any unlabelled AuNCs or reagent. The optimum concentration of each primary antibody was experimentally determined by an indirect immunoassay with fluorescence detection using Alexa Fluor Plus (A32731 and A32814), which contained Alexa® 488:goat antirabbit IgG and Alexa® 488:donkey antigoat IgG as secondary antibodies for detecting anti-h-MT2A and anti-h-APOE. For such purpose, different concentrations of each primary antibody were tested according to the recommendations of each commercial brand: 10, 5, and 2 µg mL^{-1} were the concentrations assayed for anti-h-MT2A and 20, 10, and 5 µg mL^{-1} for anti-h-APOE. The concentrations finally selected were 5 µg mL^{-1} and 10 µg mL^{-1} for anti-h-MT2A and anti-h-APOE, respectively.

HRPEsv cell treatment

In the **Figure S1,** an experimental design of the whole process where all the steps explained and performed in the current work is described. The steps encompass both the growth and treatment of the cells and their organisation for the quantification of both MT2A and APOE proteins in aliquots of treated and control cells.

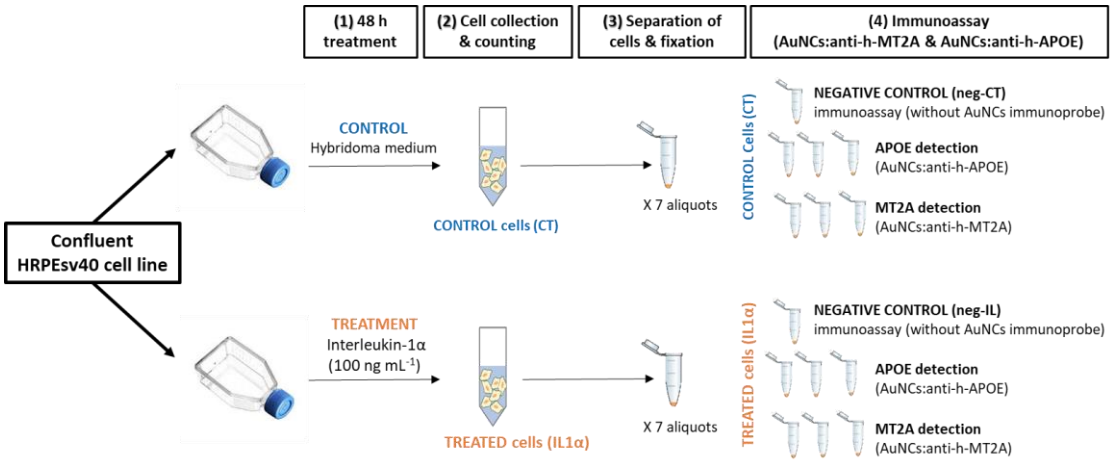


Figure S1. Schematic diagram with a summary of the experimental design performed with HRPEsv cells for the determination of MT2A and APOE proteins by sc-ICP-MS using AuNCs immunoprobes as elemental labels. The scheme includes several steps: cell treatment, cell preparation (collection, counting and fixation) and immunoassays conducted.

RESULTS AND DISCUSSIONS

Optimisation of the immunoassay

Different incubation times were assayed for the optimization of the immunoassay steps for the protein detections. Times such as the primary antibody (the metal immunoprobe) incubation was evaluated. In that vein, the immunoprobe was incubated for 1 hour at room temperature or overnight at 4ºC (as it is common in the immunocytochemistry protocols). However, overnight incubation showed high dissolved as it is demonstrated in the **Figure S2** due to the cellular breakage.

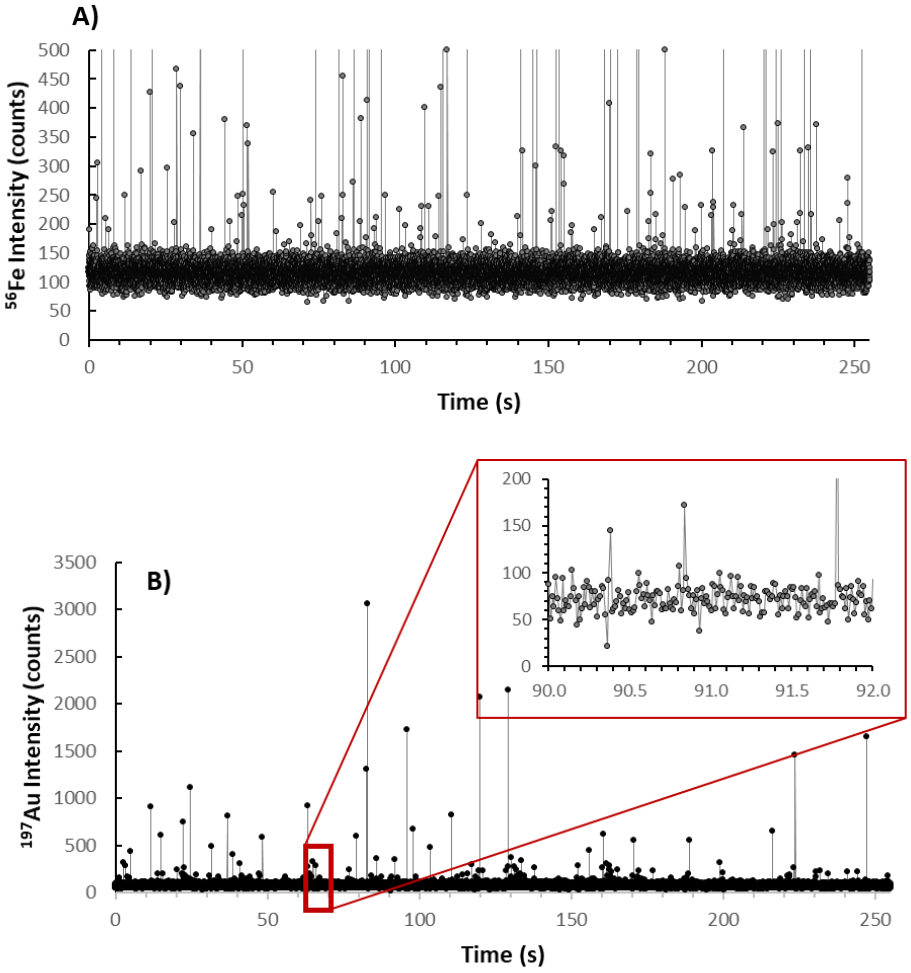


Figure S2. $^{56}Fe^{+}$ and $^{197}Au^{+}$ time-resolved profiles obtained for CT HRPEsv cells subjected to the immunoassay for the determination of APOE protein, using an overnight incubation with the AuNCs:anti-h-APOE immunoprobe. A) $^{56}Fe^{+}$; and B) $^{197}Au^{+}$. Enlarged profile shows a zoom of the background level for the $^{197}Au^{+}$ intensity signal (i.e., dissolved Au).

Determination of APOE and MT2A in individual HRPEsv cells by sc-ICP-MS

In the **Figure S3**, the steps carried out to obtain the mass of each protein is carefully explained. Taking into account the factor of amplification (atoms of gold per available antibody) obtained from the characterization of the immunoprobe, the mass of gold determined by sc-ICPMS can be transformed into fg of APOE or MT2A considering the molecular mass of each protein.

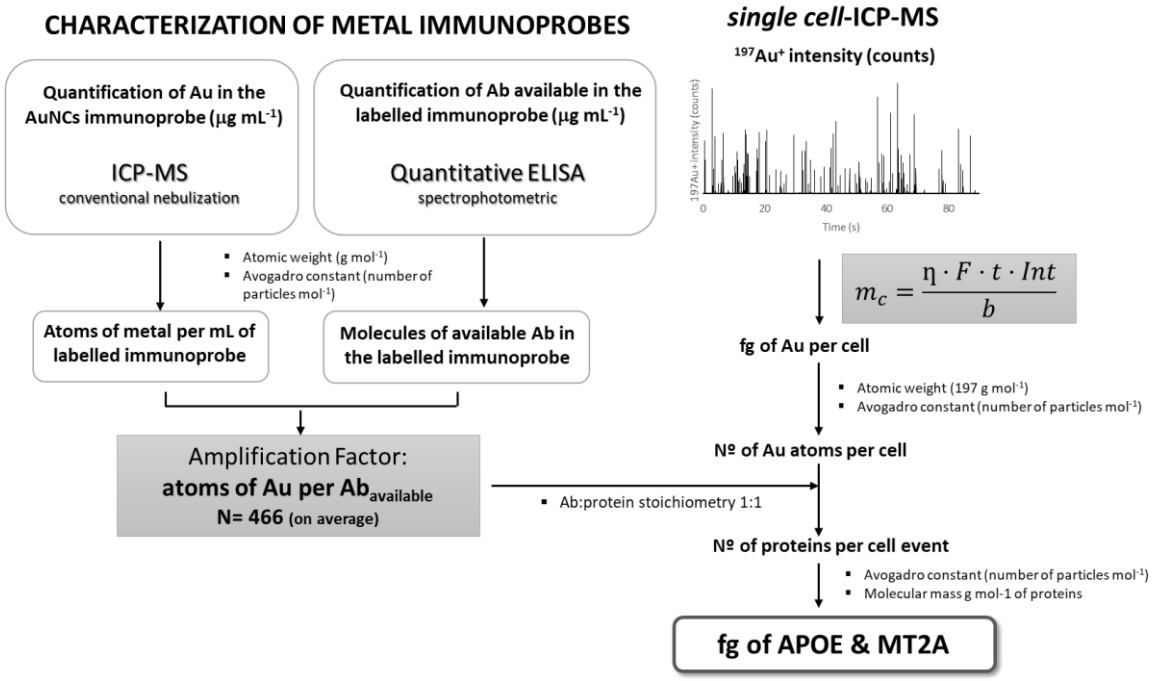
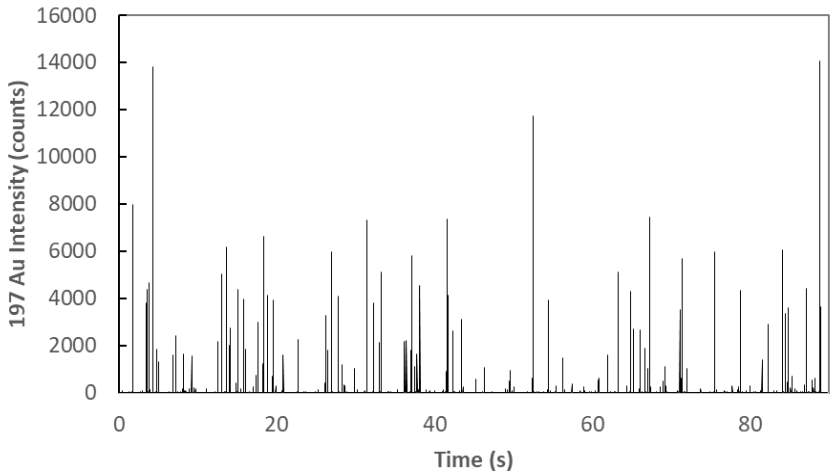


Figure S3. General diagram containing the steps required to transform $^{197}Au^{+}$ intensity signals from AuNCs immunoprobes (measured by sc-ICP-MS) into protein concentration (MT2A or APOE) present in each individual HRPEsv cell.

APOE concentration in individual HRPEsv cells: CT versus IL1α-treatment

In the **Figure S4** can be seen an example of the time profiles obtained for CT and IL1α–treated HRPEsv cells using the AuNC AuNCs:anti-h-APOE immunoprobe to evaluate the influence of the pro-inflammatory treatment with IL1α in individual cells. From this kind of time profiles the content of APOE in HRPEsv cells could be determined by sc-ICP-MS.

A)



B)

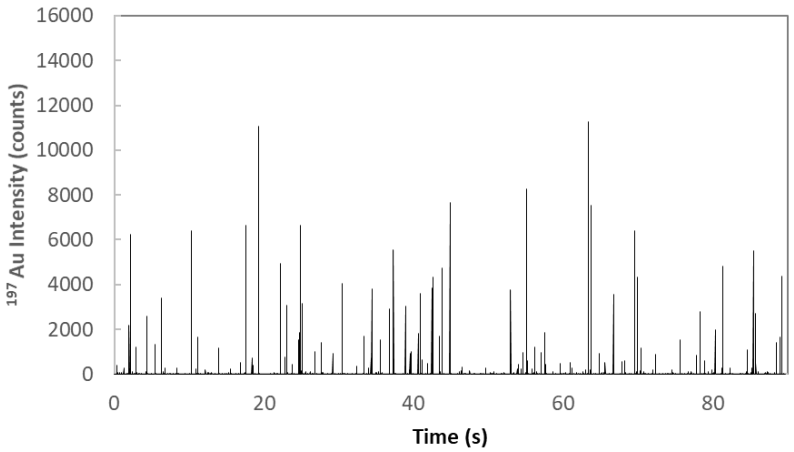


Figure S4. $^{197}Au^{+}$ time-resolved profile obtained for the analysis of APOE protein in HRPEsv cells (AuNCs:anti-h-APOE immunoprobe) by sc-ICP-MS. A) CT cells; B) IL1a–treated cells (100 ng mL^{-1}; 48 h).

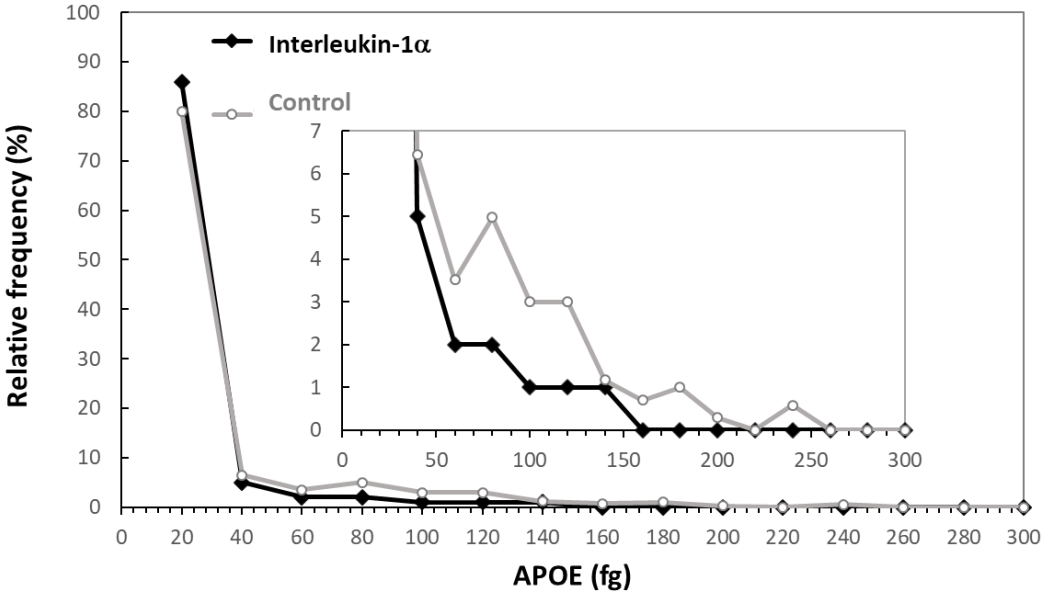


Figure S5. Mass frequency histogram for APOE protein in CT (CT2-R1; grey line) and IL1a–treated (IL3-R3; black line) HRPEsv cells obtained by sc-ICP-MS analysis. AuNCs:anti-h-APOE immunoprobe was employed for the analysis of APOE protein in HRPEsv cells.