



Development of a theranostic system for the treatment of inflammatory-based diseases



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- Inflammation is a physiological response of our body, but it has been demonstrated that it can also lead to different diseases including, among others, atherosclerosis, neurodegenerative disorders and cancer [1, 2].
- VCAM-1 is an adhesion molecule upregulated during the initial step of the formation of the atherosclerotic plaque, allowing the accumulation of leukocytes in the expanding lesions. VCAM-1 is also aberrantly expressed in different types of tumors, such as gastric, melanoma, ovarian, breast, and colorectal cancer [3].
- We conceived a three-step sequential treatment exploiting the well-known biotin/avidin affinity (Fig. 1). The first step involves NAMP, a new molecule we synthesized and characterized (Figs. 2 and 3), based on a biotin derivative linked to a VCAM-1 binding peptide. The following steps consist in the injection of avidin followed by a biotinylated tracer for PET imaging, or a biotinylated nanocarrier, to deliver drugs to the inflammation sites.

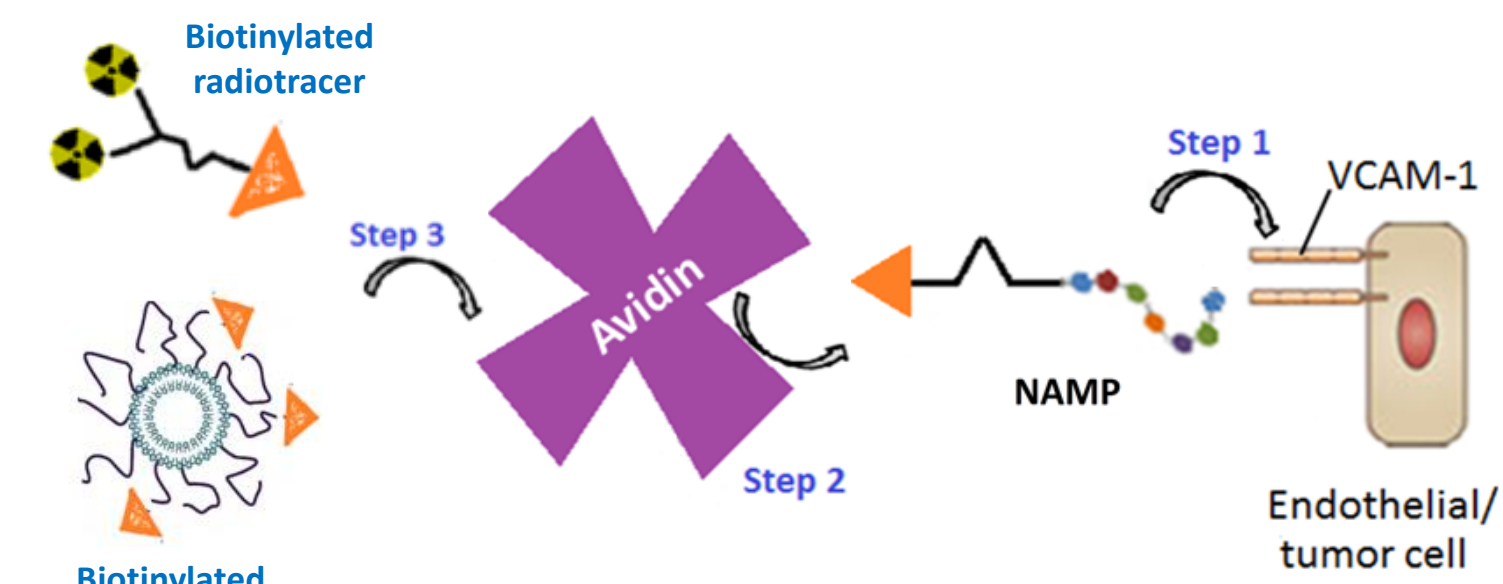


Fig. 1 Theranostic three-step pretargeting scheme

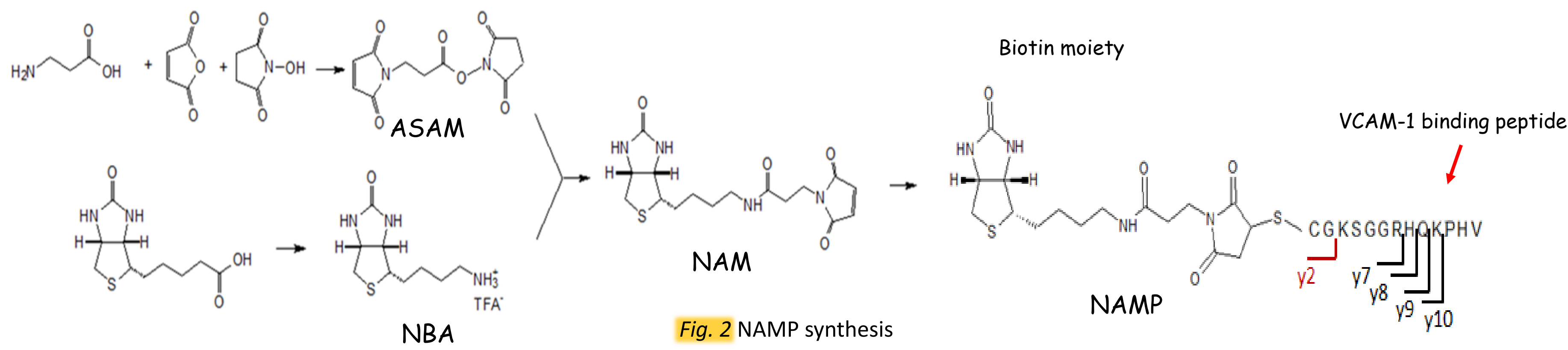


Fig. 2 NAMP synthesis

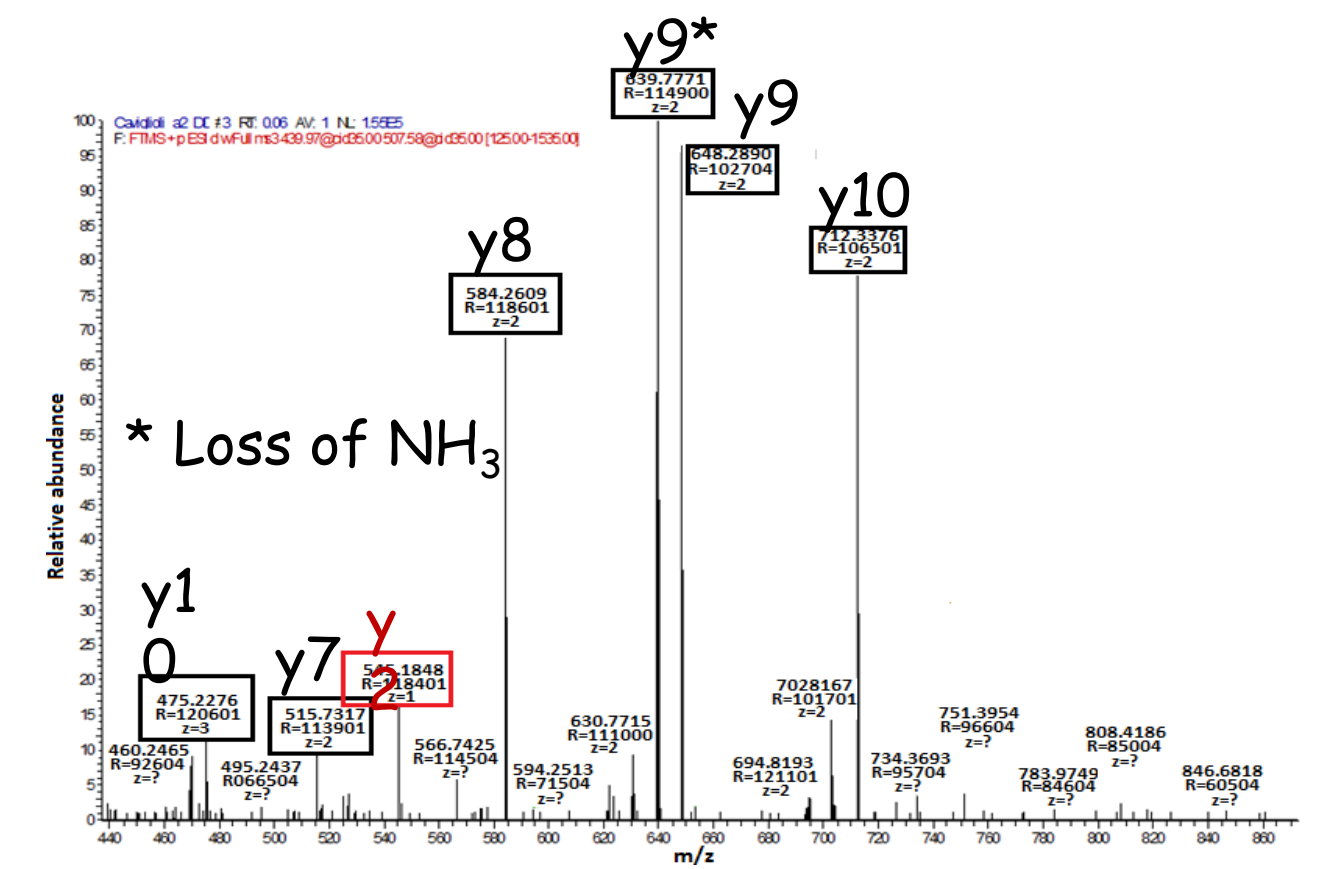


Fig. 3 NAMP ms³ fragmentation spectrum

Imaging application: As a PET tracer we selected a double chelating compound (Fig. 4), including a biotin moiety, that was labeled with ⁶⁸Ga.

Therapeutic application: Among the various nanocarriers, we selected liposomes.

Liposome preparation: Phospholipids (DSPC, cholesterol, DSPE-PEG₂₀₀₀ and DSPE-PEG₂₀₀₀-biotin) and cholesterol are dissolved in chloroform and after solvent evaporation the lipidic film is hydrated with Hepes (sodium salt) buffer. The suspension is extruded through polycarbonate filters of 400, 200 and 100 nm pore diameter to obtain LUVs (large unilamellar vesicles). For *in vitro* tests, a fluorescent dye (CM-Dil) is added to the phospholipid mixture before solvent evaporation. We statistically demonstrated that there was no significant difference in size and zeta potential between the liposomes with and without the dye.

The liposomal suspension is purified by gel filtration through a Sephadex G-50 column.

Liposome characterization: The size of liposomes is determined using PCS (photon correlation spectroscopy) and their zeta potential through electrophoretic mobility by laser Doppler micro-electrophoresis (Fig. 5). Liposome concentration, measured with a Zetasizer Ultra, was 114*10¹¹ particles/mL.

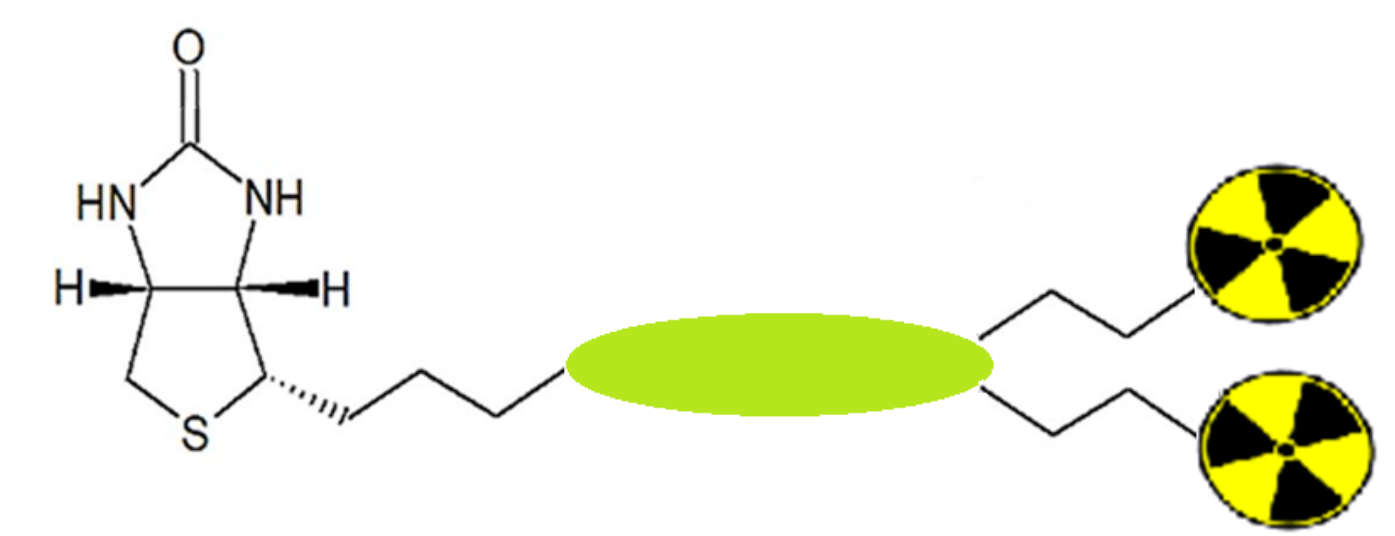


Fig. 4 General structure of a biotinylated double-chelating compound

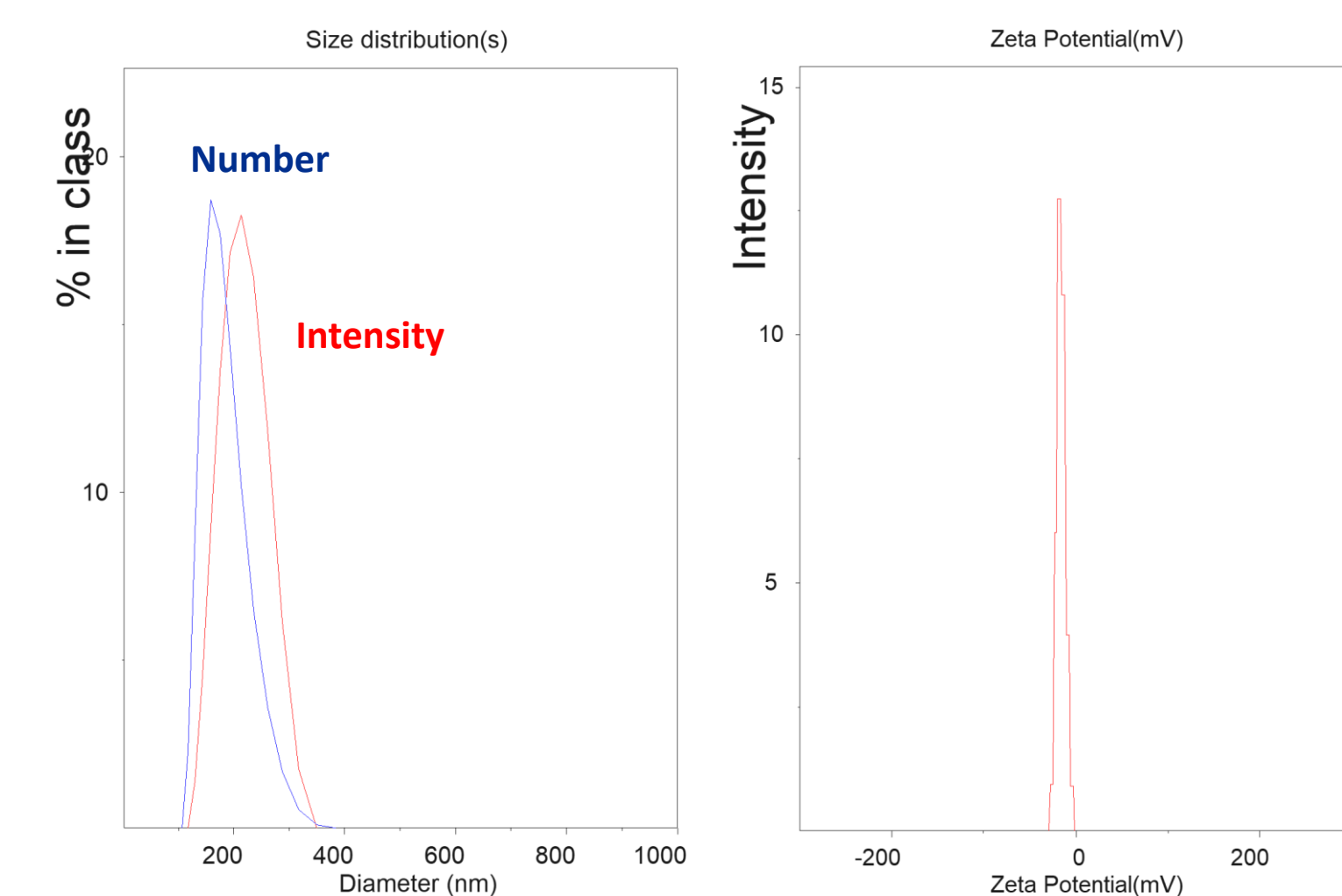


Fig. 5 On the left: liposome size distribution by intensity (red) and by number (blue); on the right, zeta potential analysis

IN VITRO CELL TESTS

Human umbilical vein endothelial cells (HUVEC) were stimulated *o/n* with TNF- α for the expression of VCAM-1, then incubated with NAMP and avidin, before being exposed to 6 MBq of ⁶⁸Ga-radiotracer; unstimulated cells were used as controls. The signal, measured with LigandTracer[®], increased with time only for activated cells (Fig. 6).

Liposomes were tested on HUVEC applying the three-step protocol (NAMP-avidin-biotinylated liposomes). The preliminary results of FACS analysis are presented in Fig. 7. The presence of NAMP seems to promote liposome internalization on TNF- α stimulated HUVEC, considering that the reduction in the fluorescence intensity could be explained by the dissociation of CM-Dil from the liposome membrane and consequent release in the aqueous cellular compartment. In fact, it is well known that these lipophilic carbocyanines are weakly fluorescent when dissolved in water, and they show fluorescence only when incorporated in membranes. Further tests, using a different fluorescent dye to label the liposomes, are needed.

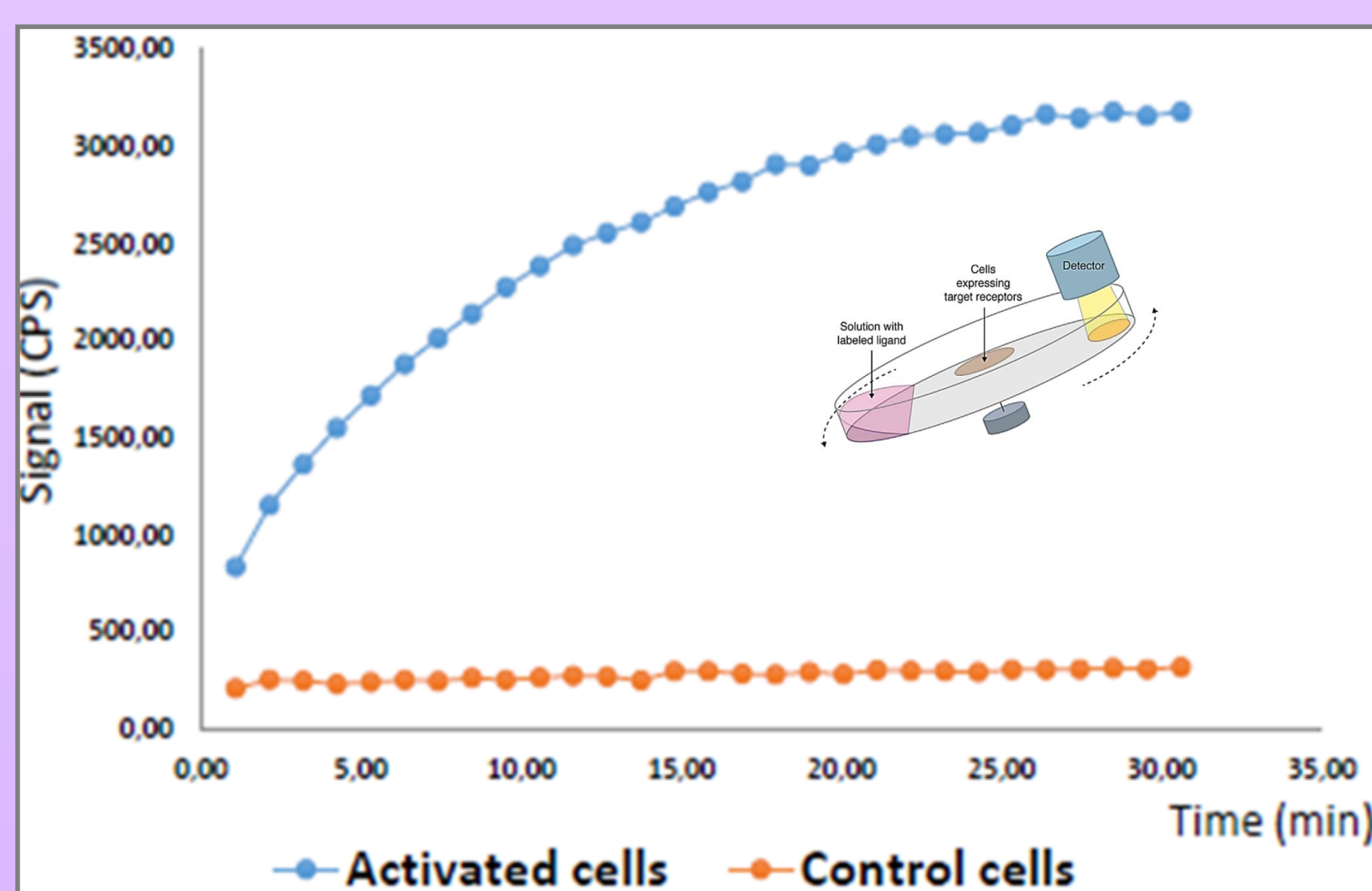


Fig. 6 Radioactivity uptake by stimulated (blue) and unstimulated (orange) HUVEC cells.

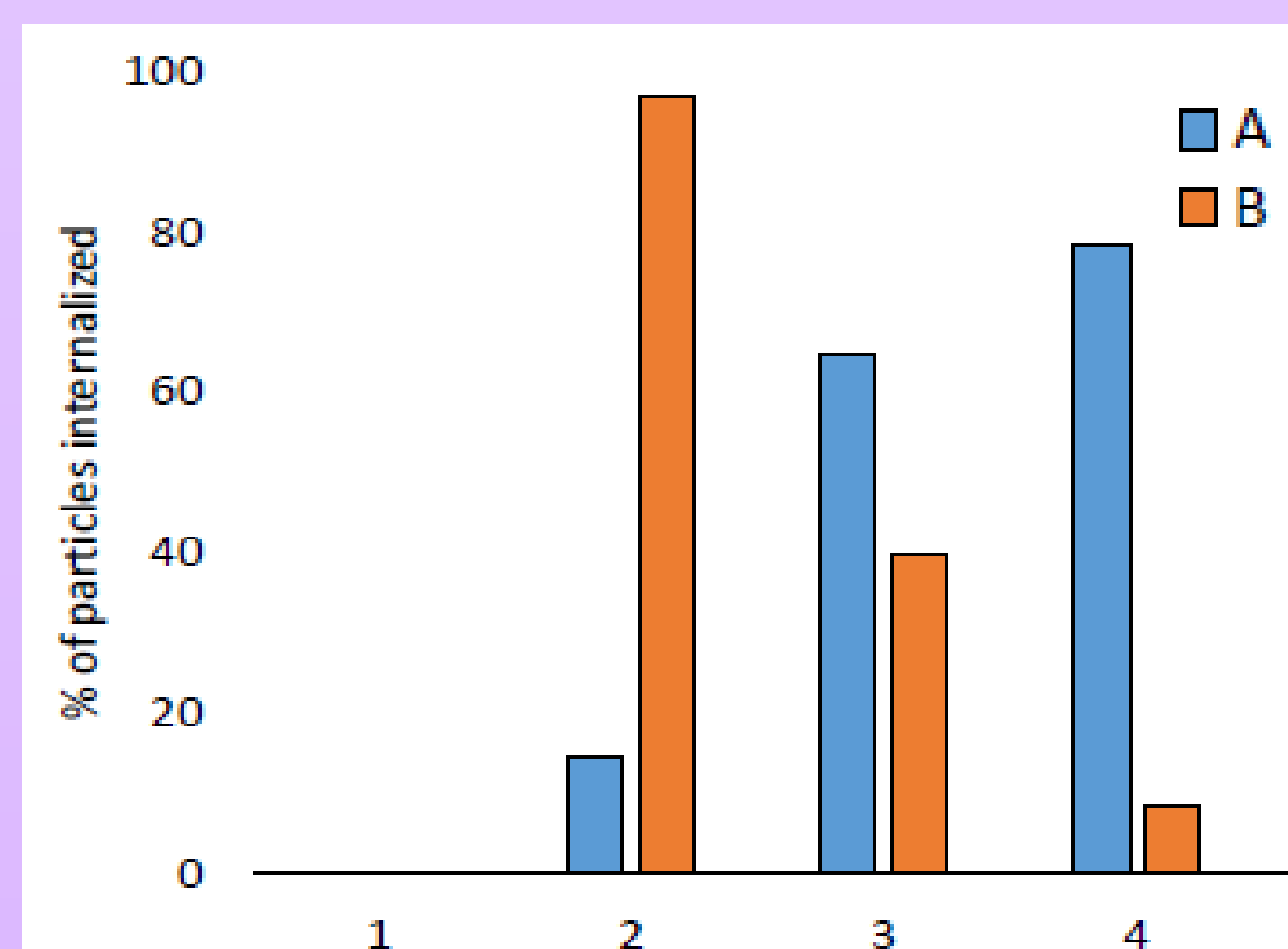


Fig. 7 FACS results: A (blue) indicates non-activated cells, B (orange) are cells activated with TNF- α (4 h, 37 °C). 1: negative controls (plain cells); 2: positive control (cells treated with anti-VCAM-1 antibody); 3: cells receiving neutravidin followed by liposomes; 4: cells receiving the complete three-step pretargeting system, first NAMP, followed by neutravidin and liposomes.

CONCLUSIONS

In vitro cell tests demonstrated that NAMP retained the capability of binding VCAM-1, allowing the success of the three-step pretargeting system both in a diagnostic and in a therapeutic model. The possibility of a two-step pretargeting (Fig. 8), involving preliminary *in vitro* formation of the NAMP-avidin complex, is being investigated.

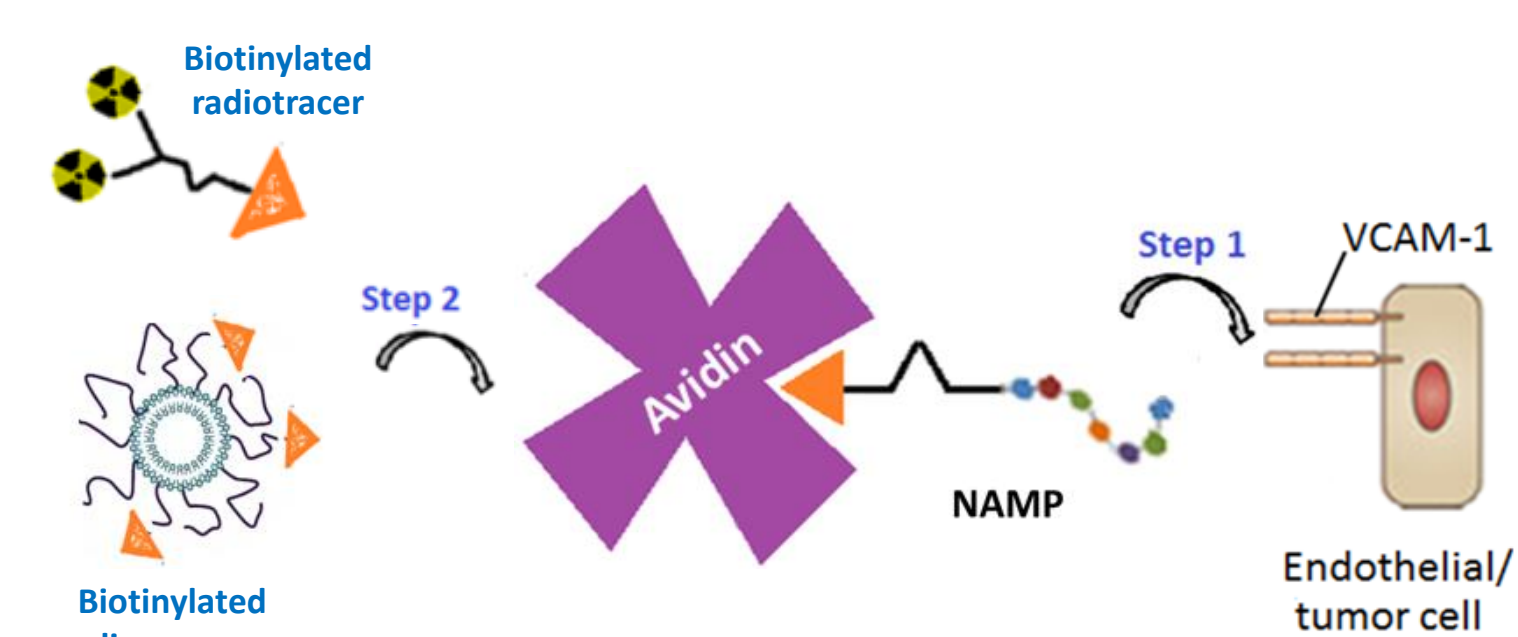


Fig. 8 Theranostic two-step pretargeting scheme

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