# Smart-design of universally decorated nano-particles for drug delivery applications driven by active transport

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**Abstract**

Targeting the cell nucleus remains a challenge for drug delivery. Here we present a universal platform for smart design of nano-particles (NPs) decoration that allows recruitment of multiple dynein motors to drive their active motion towards the nucleus. The uniqueness of our approach is based on using: (i) a spacer polymer, commonly Biotin-Polyethylene-glycol-thiol (B-PEG-SH), whose grafting density and molecular

and $\zeta$-potential measurements, respectively. All measurements are done in a temperature-controlled chamber at 25 °C ($\pm$ 0.05 °C); for data analysis the viscosity is taken as that of water (0.8872 $cP$). The NPs hydrodynamic diameter $D_h$ is extracted as follows: the intensity size distribution is extracted from the intensity auto-correlation function calculated using an ALV/LSE 5003 correlator over a time window of 30 sec (10 runs of 3 sec), repeated 3 times, using the software CONTIN. For $\zeta$-potential measurements, the solution is transferred to a U-tube cuvette (DTS1070, Malvern, England); the instrument is operated in automatic mode. Utilizing the measured NPs electrostatic mobility, the $\zeta$-potential value is determined by applying the Henry equation.[85] The $D_h$ and $\zeta$-potential values are averaged over 3 independent experiments; error bars correspond to the standard deviations of experimental values.

**UVVis absorption experiments.** UVVis absorption experiments are used to determine the mean number of bound PEG-NLS molecules per NP, $\langle N \rangle$. Analysis of the data is done using an extinction coefficient that we extract from the absorbance of solutions of increasing TAMRA-NLS concentrations that we fit using Beer-Lambert law. The absorbance is measured at a wavelength of $\lambda = 553\ nm$ resulting in an extinction coefficient $\epsilon_{553} = 0.0639$ $[1/(\mu M cm)]$.

**Western blot experiments**. WB is used to confirm the recruitment of dynein motors by the PEG-NLS coated NPs and to quantify the amount of *mammalian* dynein molecules recruited to the NP surface. The NPs are prepared as detailed above, then pelleted at 6800 $g$ for 10 $min$ at 25 °C, resuspended in a 1× Laemmli Sample Buffer, and boiled for 5 $min$ to promote the detachment of the bound proteins. The proteins are separated by electrophoresis using a 12 % agarose gel and transferred to a nitrocellulose membrane. The membrane is incubated for 1 $h$ in a blocking buffer of PBST (PBS supplemented with 0.1 $v/v$ % Tween) and 10% ($v/w$) dry skim milk (Sigma-Aldrich, St. Louis, MO, USA). The membrane is washed three times with PBST for 5 $min$ and then incubated for 1 $h$ at 25 °C with a primary anti-dynein antibody (sc-13524) (Santa Cruz Biotechnology, Dallas, TX,

USA) diluted 1:200 ($v/v$) in blocking buffer prior use. Next, the membrane is washed three times with PBST and incubated for 1 h at 25 °C with an anti-mouse HRP conjugated with a secondary antibody (sc-2005, Santa Cruz Biotechnology, Dallas, TX, USA) diluted 1:10,000 ($v/v$) in PBST supplemented with 0.5% ($v/w$) skim milk. To finalize the procedure, the membrane is washed three times with PBST and incubated with an ECL Western blot reagent (1,705,060, Bio-Rad, Hercules, CA, USA) for 5 *min* in the dark. Images are collected by chemiluminescence using a Fusion FX imaging system (Vilber Lourmat, Collégien, France). The integrated signal measured from the area of the dynein bands is extracted using ImageJ and calibrated using known masses of purified dynein protein (CS-DN01, Cytoskeleton, Denver, CO, USA) that serve as standards. A molecular weight of 2.17 $MDa$[86] is then used to derive the number of dynein motors recruited to the NPs. The standards and NP samples are prepared under identical conditions.

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