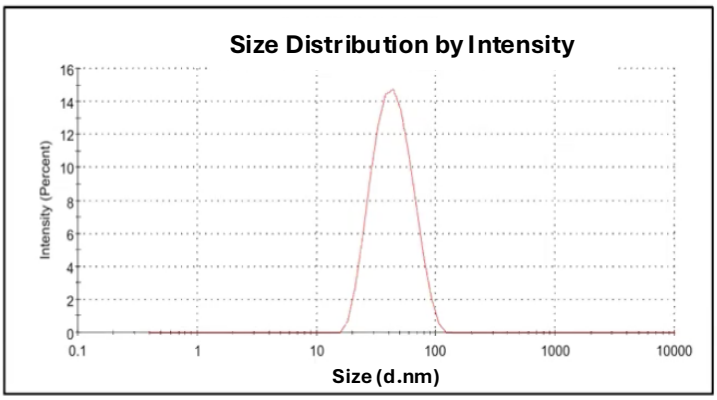
Paretor
155 Arlington St
Watertown, MA 02478

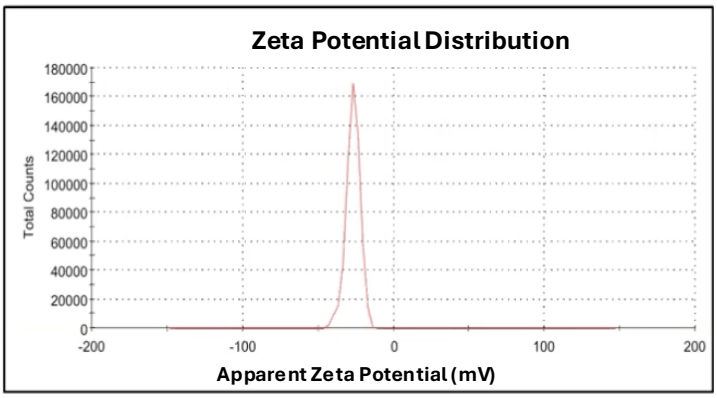
Paretor – Predictable Delivery Protocols & Formulations

Mineral Nanoparticle Colloid | Cell transfection | Cargo Carrier

Dynamic light scattering experiments show a single peak with normal distribution*



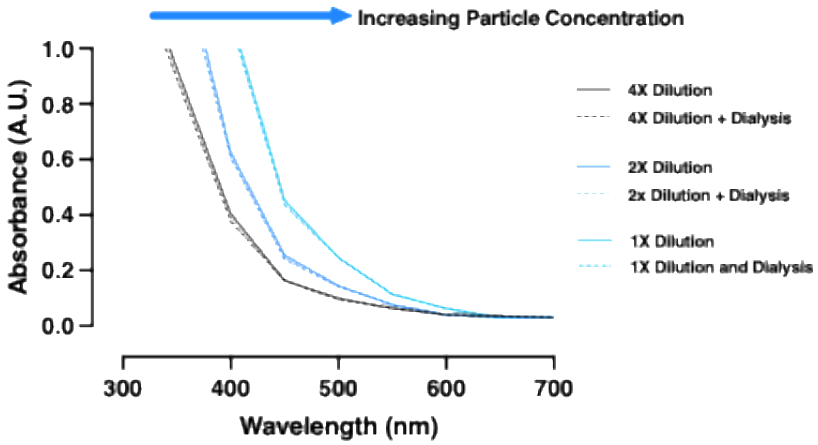
This is a single peak with a negative charge*



*Crude particles taken directly from the reactor

- Particles are uniform in size and charge
- Particles are not magnetic
- Straightforward to modify post-synthesis

Preservation of Paretor MNC Optical Profile with Dialysis



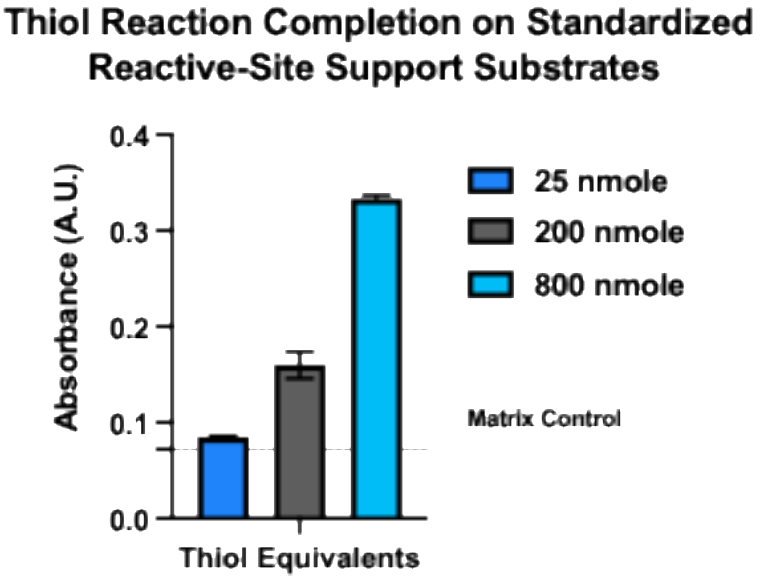
Interpretation: Paretor mineral nanoparticle colloids remain structurally intact and do not release diffusible iron under buffer exchange conditions.

Dialysis against PBS does not alter the absorbance profile, indicating the signal is dominated by particle-associated iron and diffusible iron species are minimal under these conditions. Data suggest MNCs remains structurally intact and does not release diffusible iron under buffer exchange conditions. Data also suggest there are minimal free iron residuals present post synthesis. Absorbance scales with particle concentration.

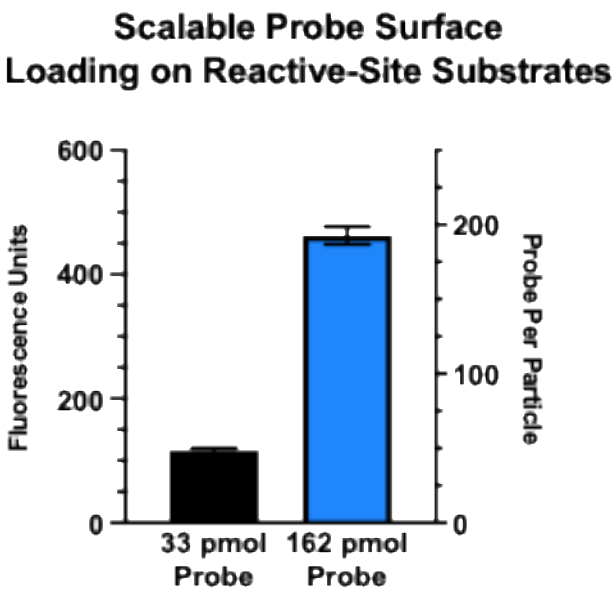
Paretor – Predictable Delivery Protocols & Formulations

Mineral Nanoparticle Colloid | Cell transfection | Cargo Carrier

Stoichiometric, Payload-Agnostic Probe Loading onto Paretor MNCs Under Single-Phase Aqueous Conditions



One 4 x 4 mm TCEP substrate supports the activation of 800 nmol thiol equivalents (Cysteine, DNTB)



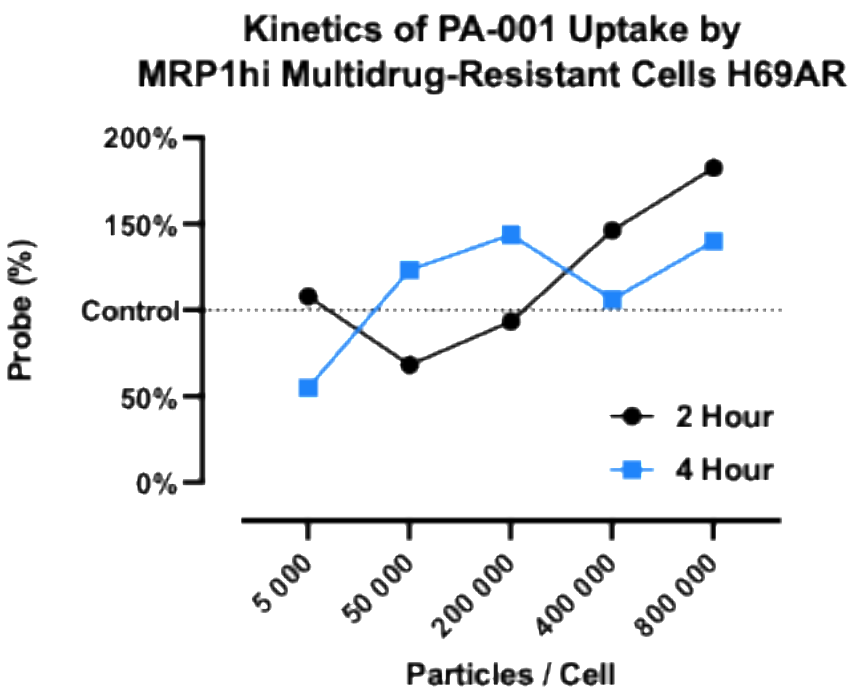
Scalable probe surface loading on reactive-site substrates. Labeled PA-001 was quantified by fluorescence intensity.

- Non-crystalline stabilization of PA-001 enables post-synthesis surface functionalization via the direct binding of iron-coordinated reactive sites:
  - Phosphonate : Maleimide : Cys : AQuora fluorescent probe
  - 10 kDa MW cutoff dialysis cassette for easy cleanup
- Increasing probe inputs (e.g., 33 pmol vs. 162 pmol) produce a predictable, proportional increase in surface loading, consistent with stoichiometric consumption of accessible Fe(III) coordination sites
- The process requires no specialized equipment, no pre-functionalized nanoparticles, and no chromatographic purification
- These data demonstrate that Paretor colloidal nanoparticles serve as a payload-agnostic, universally modifiable interface whose carrier CMC and payload CMC remain separable, supporting robust bench-to-bedside and field-deployable functionalization

No warrantees nor guarantees of performance in specific applications.

Paretor – Predictable Delivery Protocols & Formulations

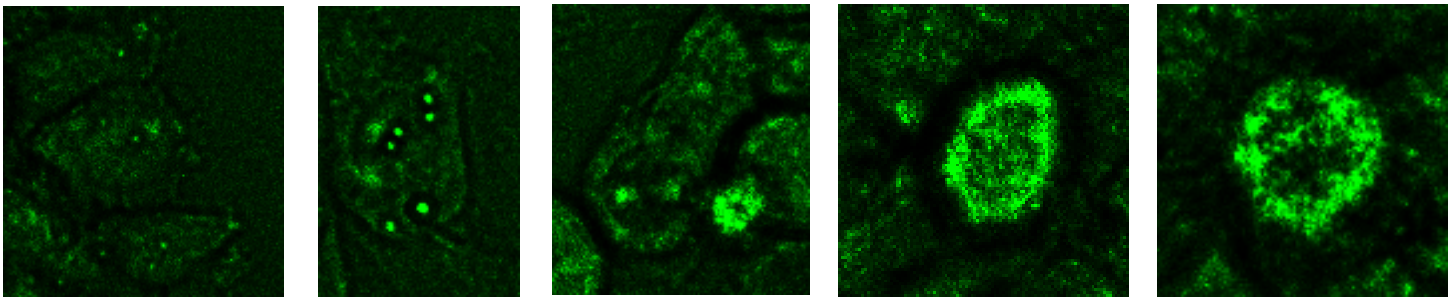
Mineral Nanoparticle Colloid | Cell transfection | Cargo Carrier



Kinetics of uptake by MRP1hi Multidrug-Resistant Cells H69AR. Data shown as percent of control. Flow cytometry data revealed that <20% of cells were transfected .Similar results seen with BT20 cells (data not shown)

Flow cytometric analysis was performed on MRP1hi H69AR multi-drug-resistant cancer cells incubated with thawed PA-001 particles at doses of 5k, 50k, 200k, 400k and 800k particles per cell. Prior to incubation at 37 °C for 2 or 4 hours, particles were activated at 50°C for 20 minutes then labeled with a thiolated AHX (high MDR liability molecule) linked to a fluorescein probe (Tracer) for an additional 20 minutes at room temperature, then dialyzed against PBS for 1 hour using a 10kDa MW cutoff membrane. Probe was visualized at a 20x magnification after washing and prior to removal. After removal and suspension in EDTA/PBS cells were analyzed by flow cytometry using a FSChi gate with side scatter (SSC-A) and fluorescein channel (BL1-A) to assess uptake and internalization. These results indicate that tracer-labeled carrier particles (10-003) engage with and or are internalized by H69AR cells

Stages of PA-001-mediated AHX uptake & efflux in H69AR cells



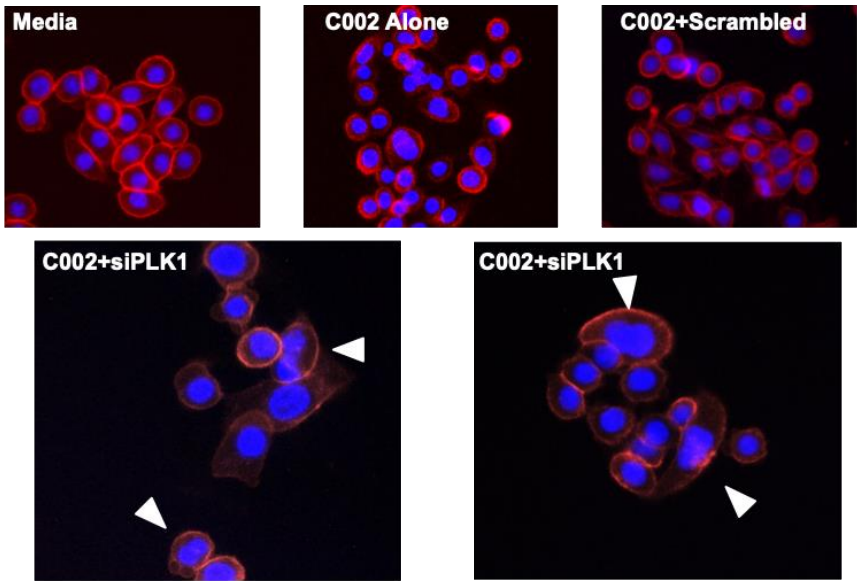
Un-transfected > Puncta > Dispersed > Cytosolic > Efflux

PA-001 post-synthetically labeled with fluorescein-based probe via thiol linkage used to transfect high multi-drug resistant (MDR) cell line H69AR. The live images were taken 4 hours post transfection and show uptake and membrane associated MRP-1 based efflux. FACS data show <20% uptake in the asynchronous cell culture.

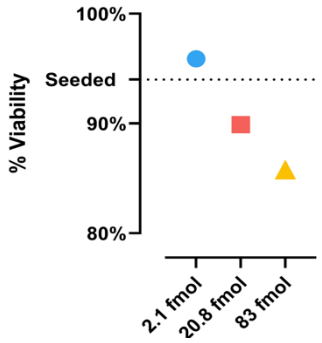
Paretor – Predictable Delivery Protocols & Formulations

Mineral Nanoparticle Colloid | Cell transfection | Cargo Carrier

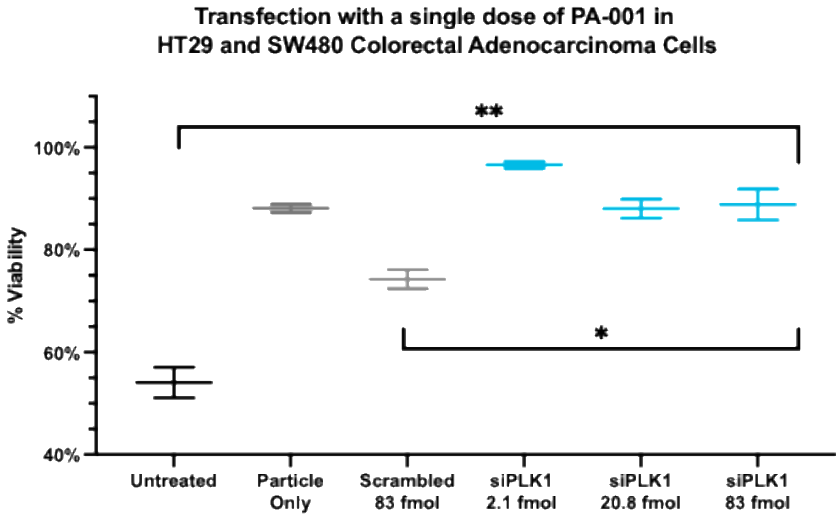
Imaging + viability analyses confirm that PA-001 nanoparticles deliver functional siRNA at femtomolar doses to colorectal cancer cells



Under single-dose femtomolar exposure conditions, PA-001 + siPLK1 produces the characteristic PLK1 silencing barbell phenotype in SW480 cells at 48 hours, while control conditions preserve baseline morphology (Left)



HT29 cells treated with PA-001-siPLK1 at 2.1, 20.8, and 83 fmol per well for 44 hours exhibited a dose-dependent reduction in viability, with nuclear morphology consistent with mitotic arrest (Above)



*Statistics: Brown–Forsythe one-way ANOVA and Welch's ANOVA indicate a significant overall effect of treatment condition; bars denote omnibus significance, not pairwise comparisons. N=2 independent measurements per condition; pooled across HT29 and SW480.

Assay controls revealed an unexpected pattern. Assay stress exposed PA-001's ability to preserve viability in HT29 and SW480 cells (Left). This finding has important implications for siRNA screening, as the particles themselves impart a viability advantage.

V1.0 04022026