

# Testing pH-triggered charge reversal in tumour-responsive carriers

Zeta-potential titration and uptake assays that confirm a carrier switches charge in mildly acidic conditions

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## Principle

Charge-shifting carriers mask surface amines with acid-labile groups such as 2,3-dimethylmaleic amide. At pH 7.4 the surface is negative or near-neutral; in the mildly acidic tumour microenvironment (pH 6.5–6.8) the amides hydrolyse and the surface becomes positive, increasing binding to tumour cells. Two measurements confirm the switch: surface charge over time at each pH, and cell uptake at matched pH.

## Zeta-potential titration

- Dilute the carrier to 0.1 mg/mL lipid in 10 mM buffers at pH 7.4, 6.8, 6.5 and 6.0 (low ionic strength).
- Incubate at 37 °C and measure zeta potential at 0, 0.5, 1, 2 and 4 h.
- Include a non-masked cationic control and a non-cleavable (succinyl) masked control.

## Uptake assay

- Label the carrier with a lipophilic dye (e.g. DiD).
- Pre-incubate the carrier for 1 h at pH 7.4 or 6.5, then add to tumour cells in medium buffered to the same pH.
- After 2 h, wash, trypsinise and read mean fluorescence by flow cytometry.

## Interpreting results

| Result                                           | Meaning                                              |
|--------------------------------------------------|------------------------------------------------------|
| Positive at pH 6.5 within 1–2 h, negative at 7.4 | Switch works in the intended window                  |
| Positive at both pH values                       | Masking incomplete or unstable; check conjugation    |
| No flip at pH 6.5                                | Linker too stable; consider a more acid-labile group |
| Uptake ratio (6.5 / 7.4) > 2                     | Charge reversal translates into cell binding         |