Development of functionalized nanocarriers for drug targeting in tuberculosis

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CRS

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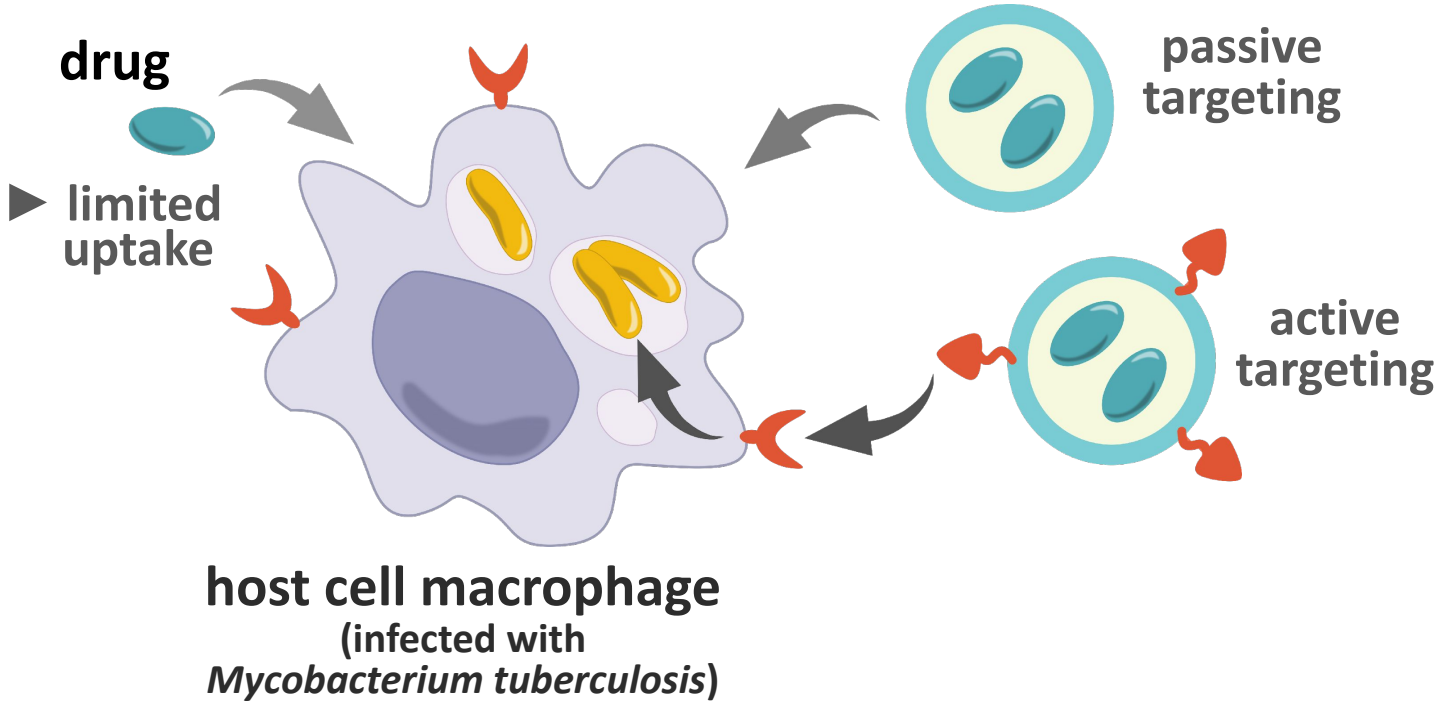
Bionanosurf

MARIE CURIE ACTIONS

INMA Instituto de Nanociencia y Materiales de Aragón

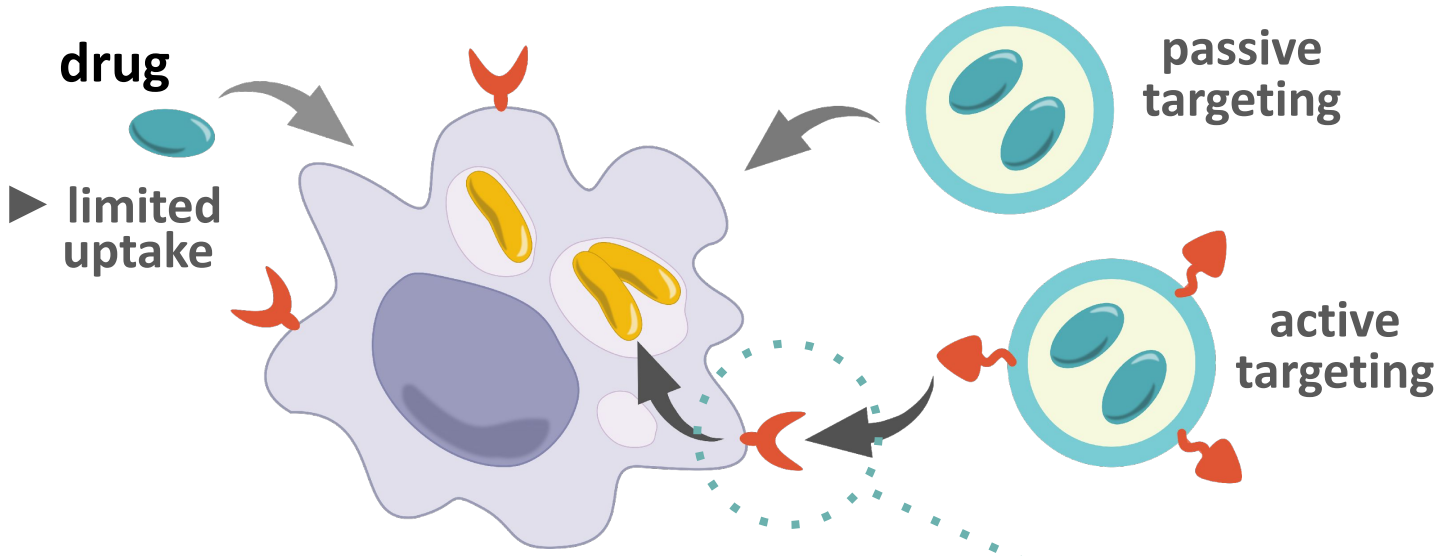
1. Concept – nanocarrier-based targeted drug delivery against tuberculosis



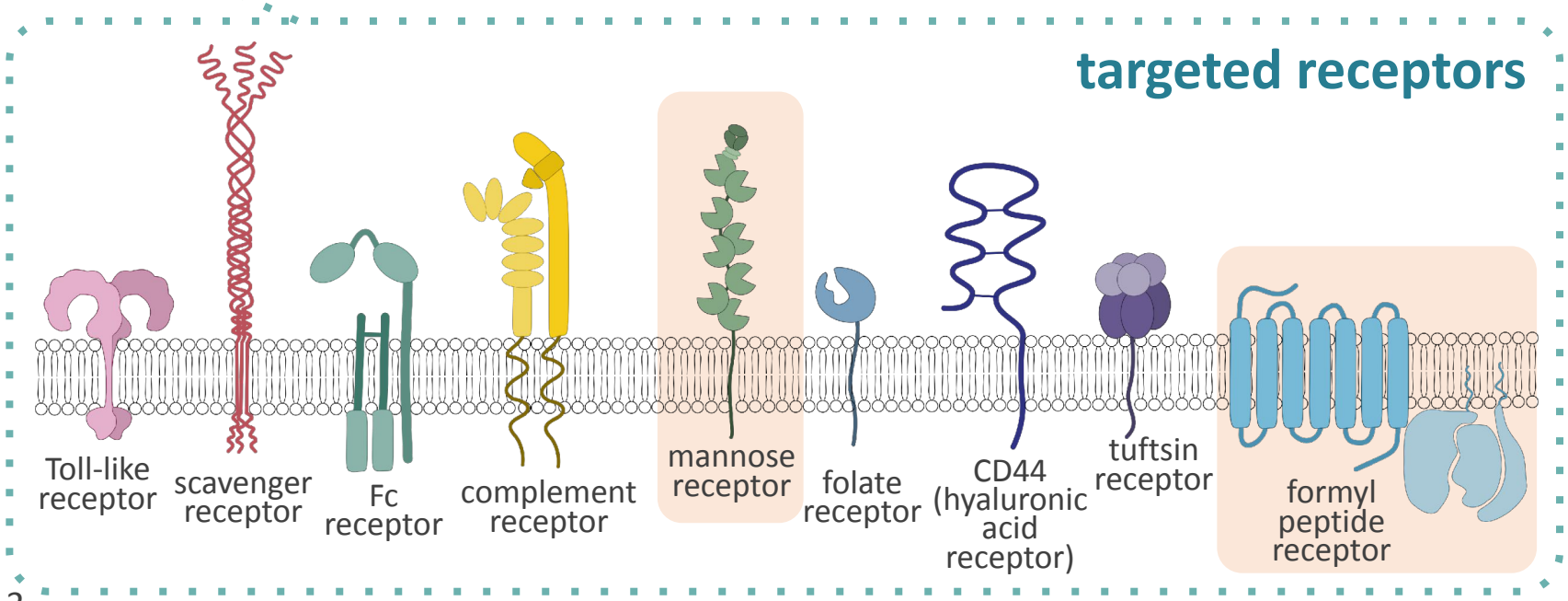
- enhanced bioavailability
- targeted delivery

Z. Baranyai et al., *Adv. Ther.*, 2021, 4, 202000113

1. Concept – nanocarrier-based targeted drug delivery against tuberculosis



- enhanced bioavailability
- targeted delivery



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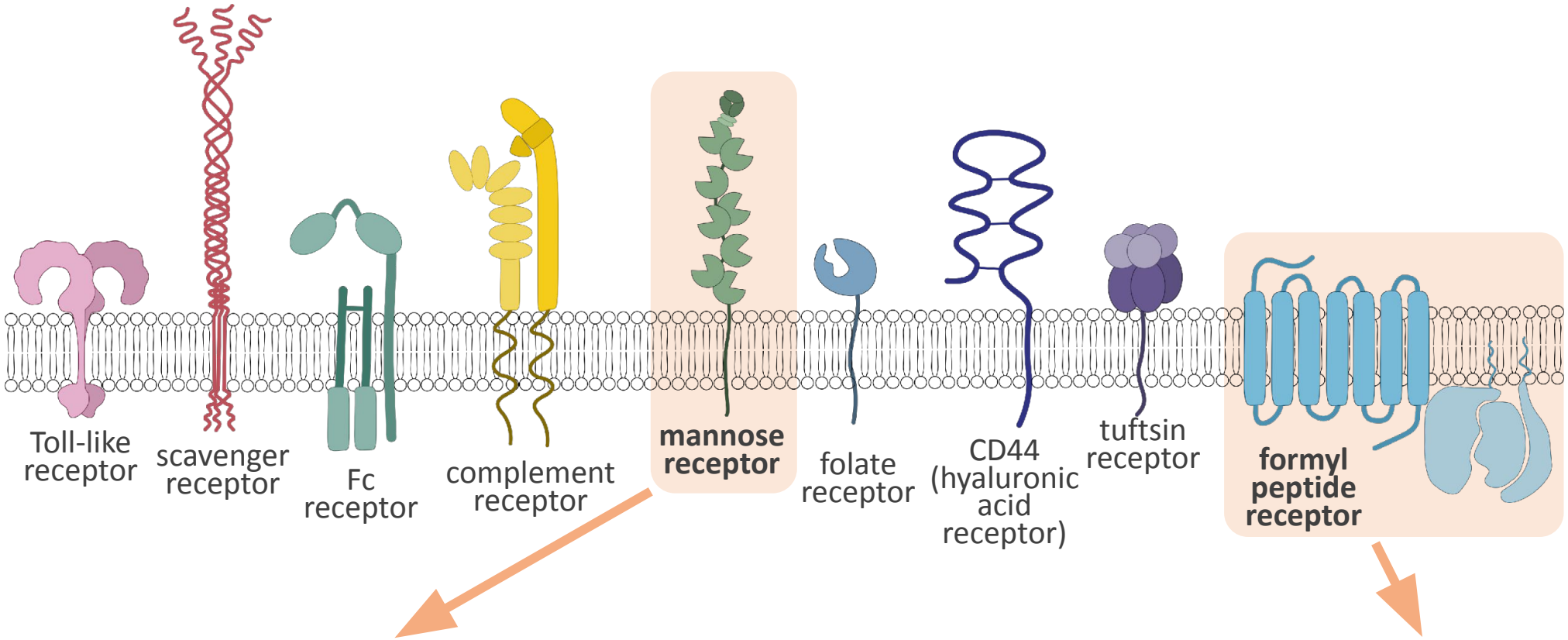
1. Concept – targeted receptors



- ligands: e.g., terminal mannose, *N*-acetylglucosamine
- uptake and intracellular survival of *M. tuberculosis*
- formation of granulomas

- ligands: e.g., formyl-methionine peptides
- chemoattractant receptors
- innate immunity

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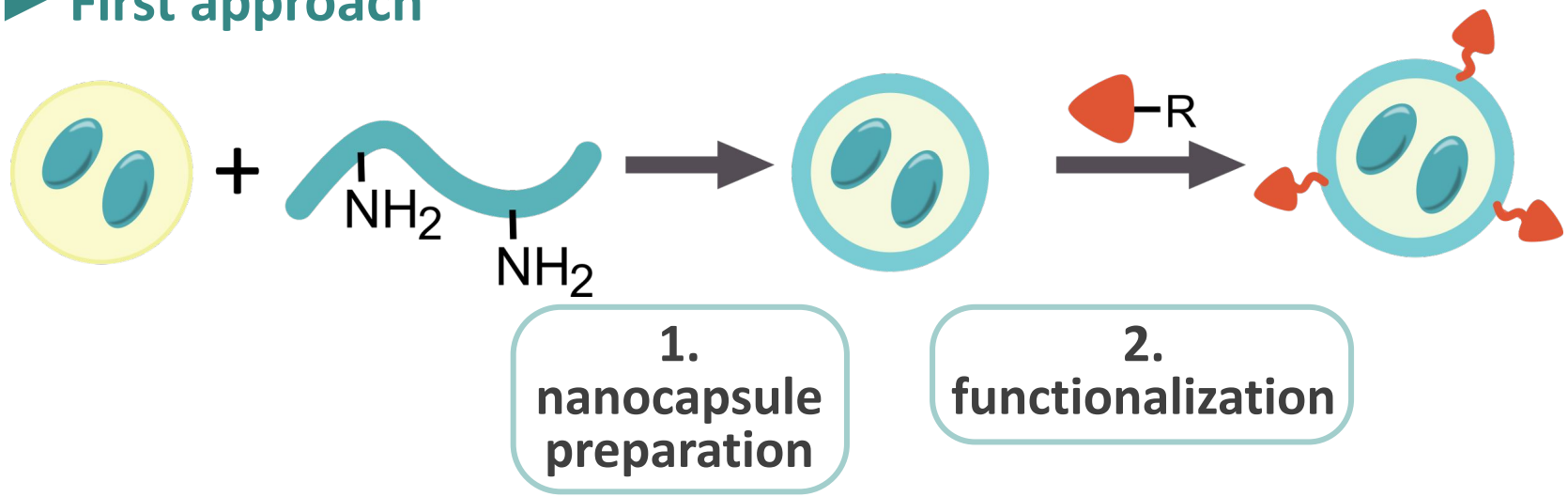
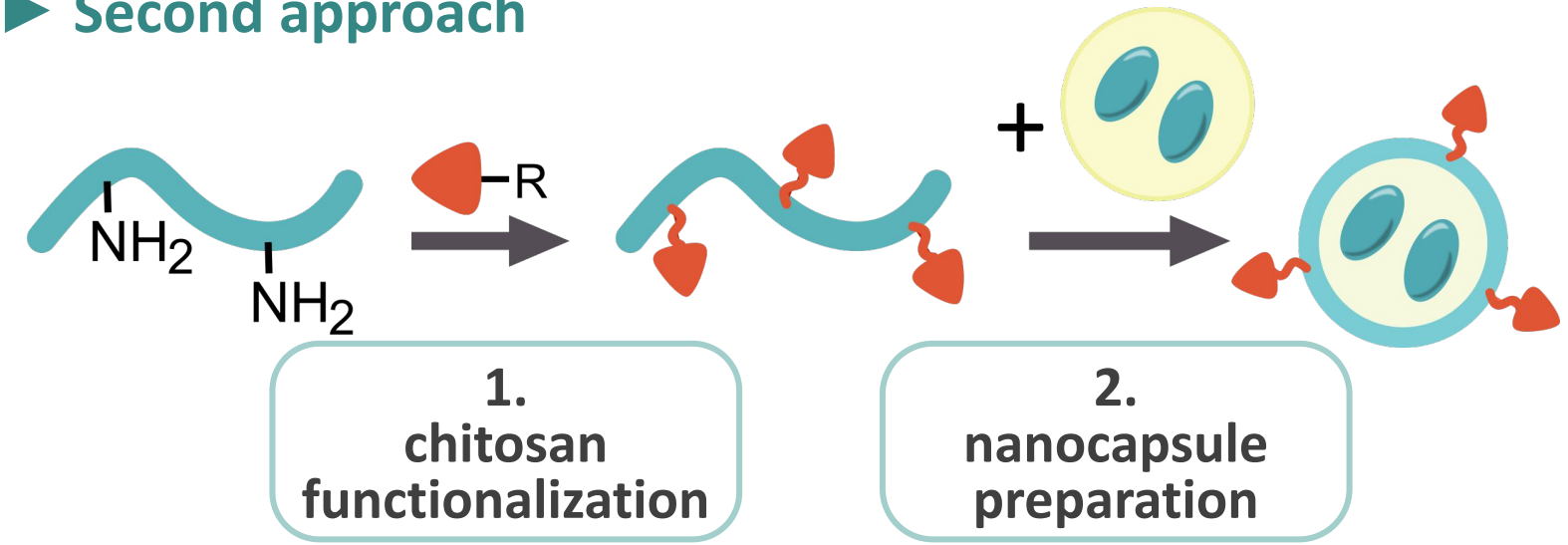
2. Preparation of functionalized chitosan nanocapsules (NCs)

▶ First approach



Problems:
- poor stability during functionalization
- low drug loading
- low yield

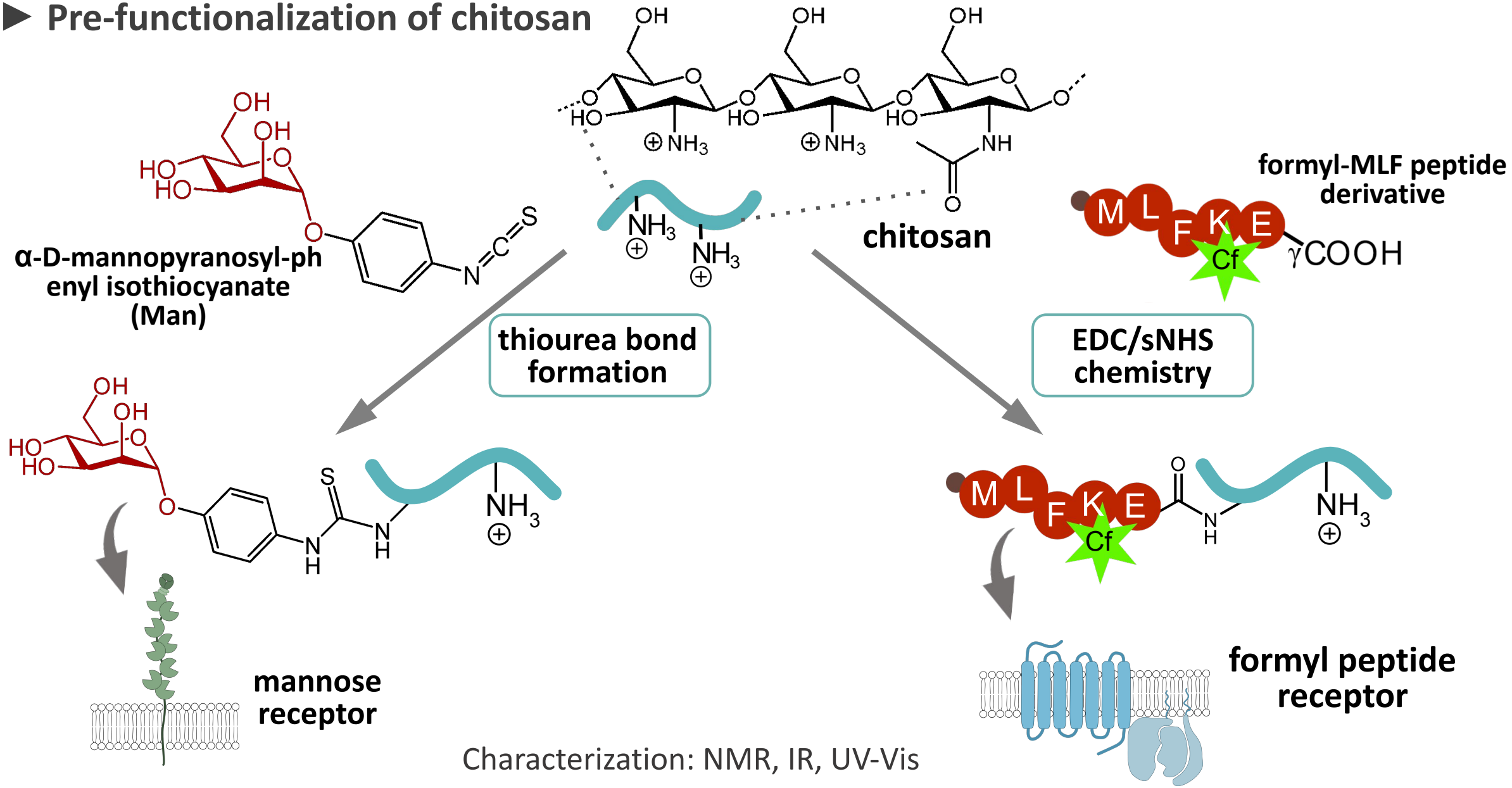
▶ Second approach



Advantages:
- improved stability
- high drug loading
- high yield

2. Preparation of functionalized chitosan nanocapsules (NCs)

▶ Pre-functionalization of chitosan



Characterization: NMR, IR, UV-Vis

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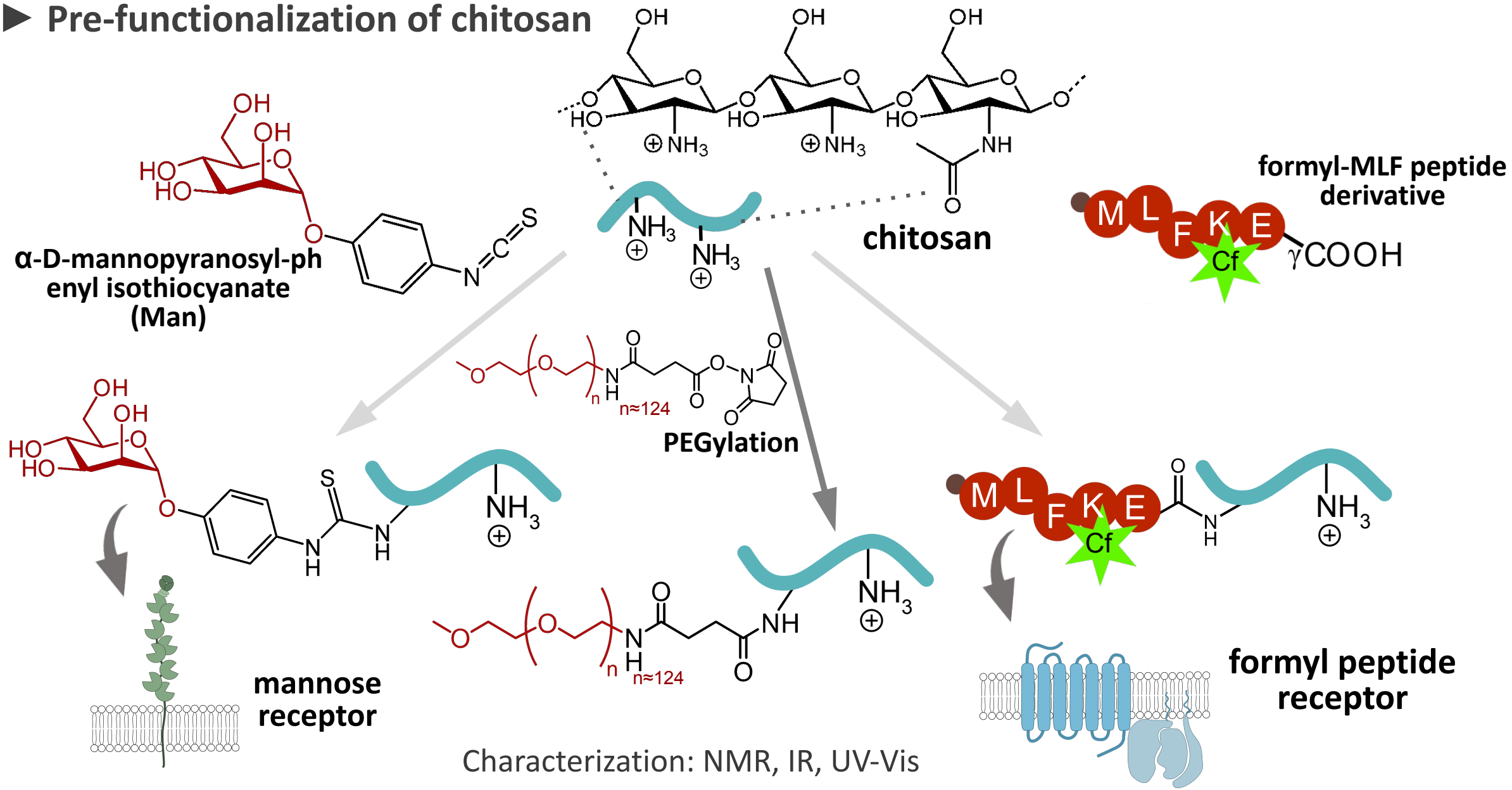
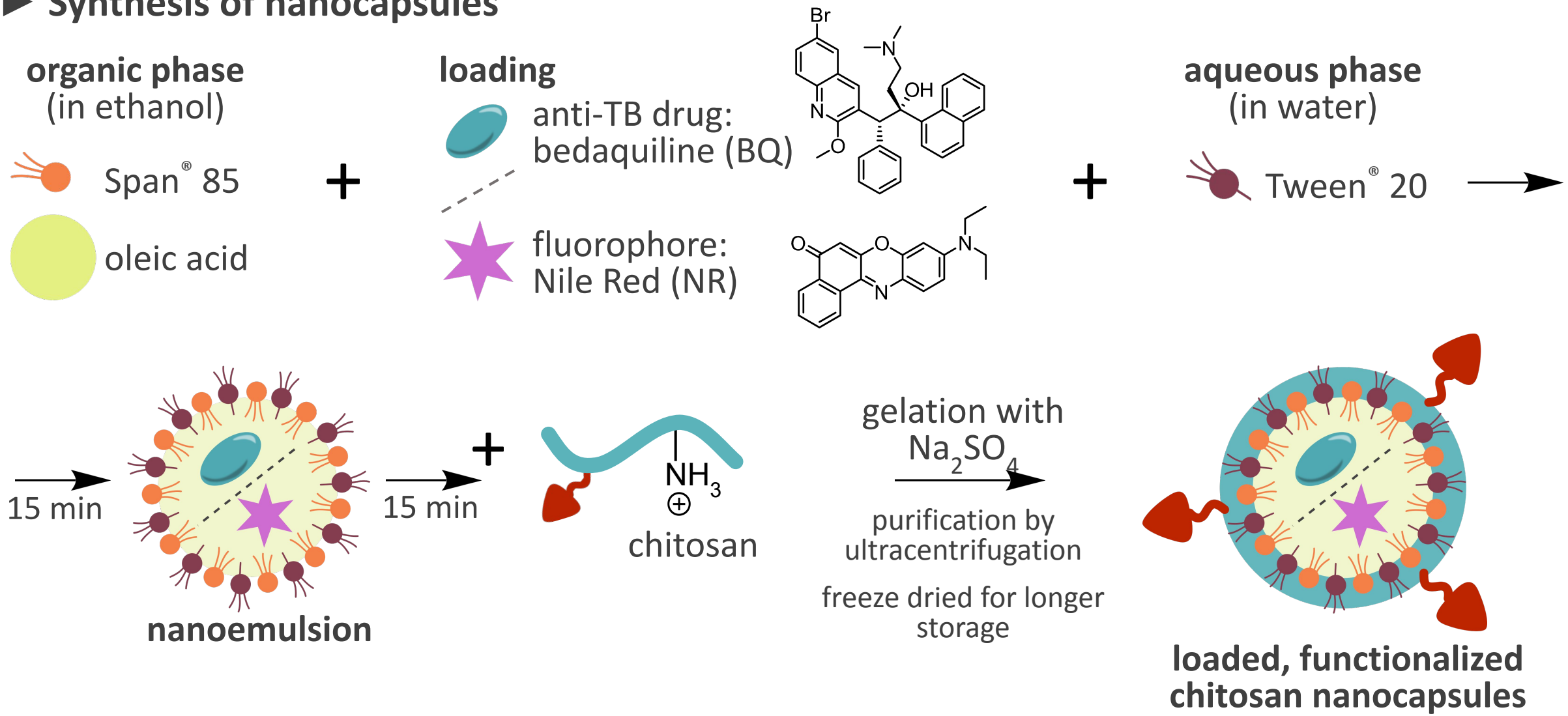


Characterization: NMR, IR, UV-Vis

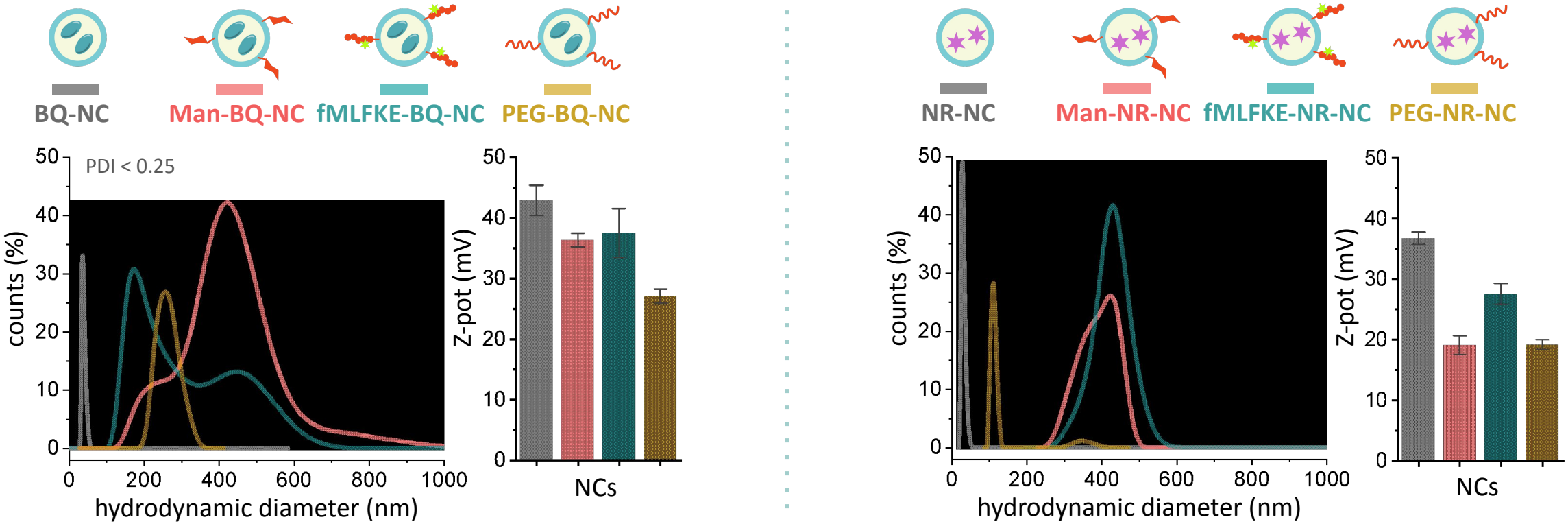
2. Preparation of functionalized chitosan nanocapsules (NCs)

- Pre-functionalization of chitosan
- **Synthesis of nanocapsules**



3. Characterization

- **Size** (DLS): 50-600 nm
- **Zeta-potential:** +20-40 mV



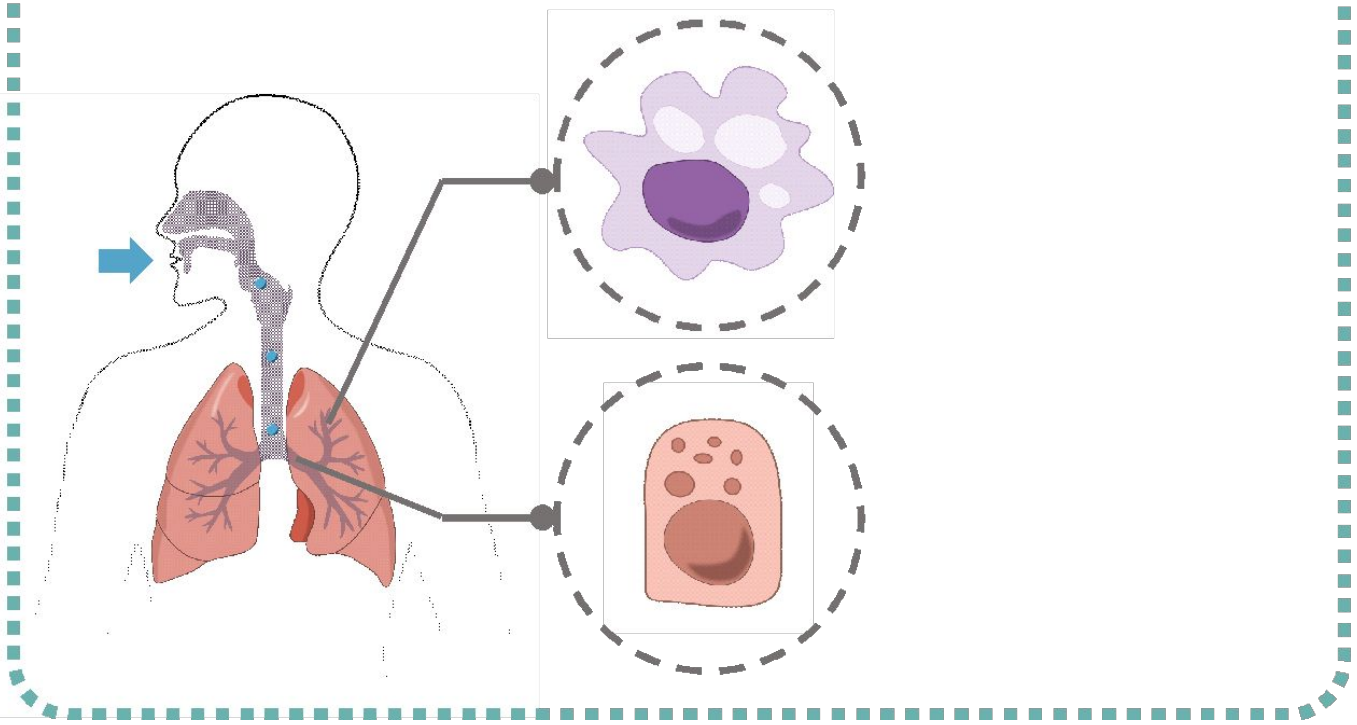
- **EE & DL**

EE: encapsulation efficiency
DL: drug loading
FL: fluorophore loading

NC	EE (%)	DL (%)
BQ-NC	76±3	32±1
Man-BQ-NC	53±1	36±1
fMLFKE-BQ-NC	66±8	26±3
PEG-BQ-NC	63±2	28±1

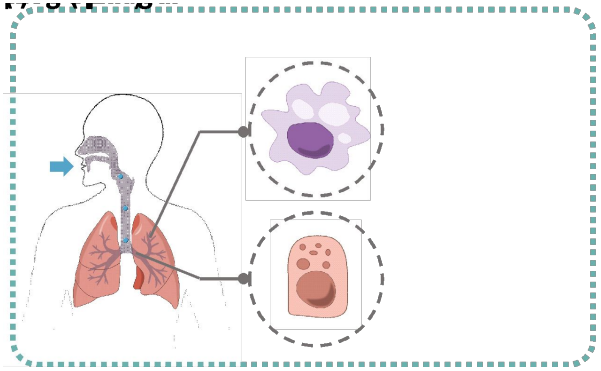
NC	EE (%)	FL (%)
NR-NC	55±3	0.044±0.002
Man-NR-NC	56±5	0.047±0.004
fMLFKE-NR-NC	71±9	0.050±0.008
PEG-NR-NC	41±2	0.047±0.002

4. *In vitro* evaluation



- **Cytotoxicity**
  - no cytotoxicity at MIC level
- **Internalization**
- **Activity against bacteria**

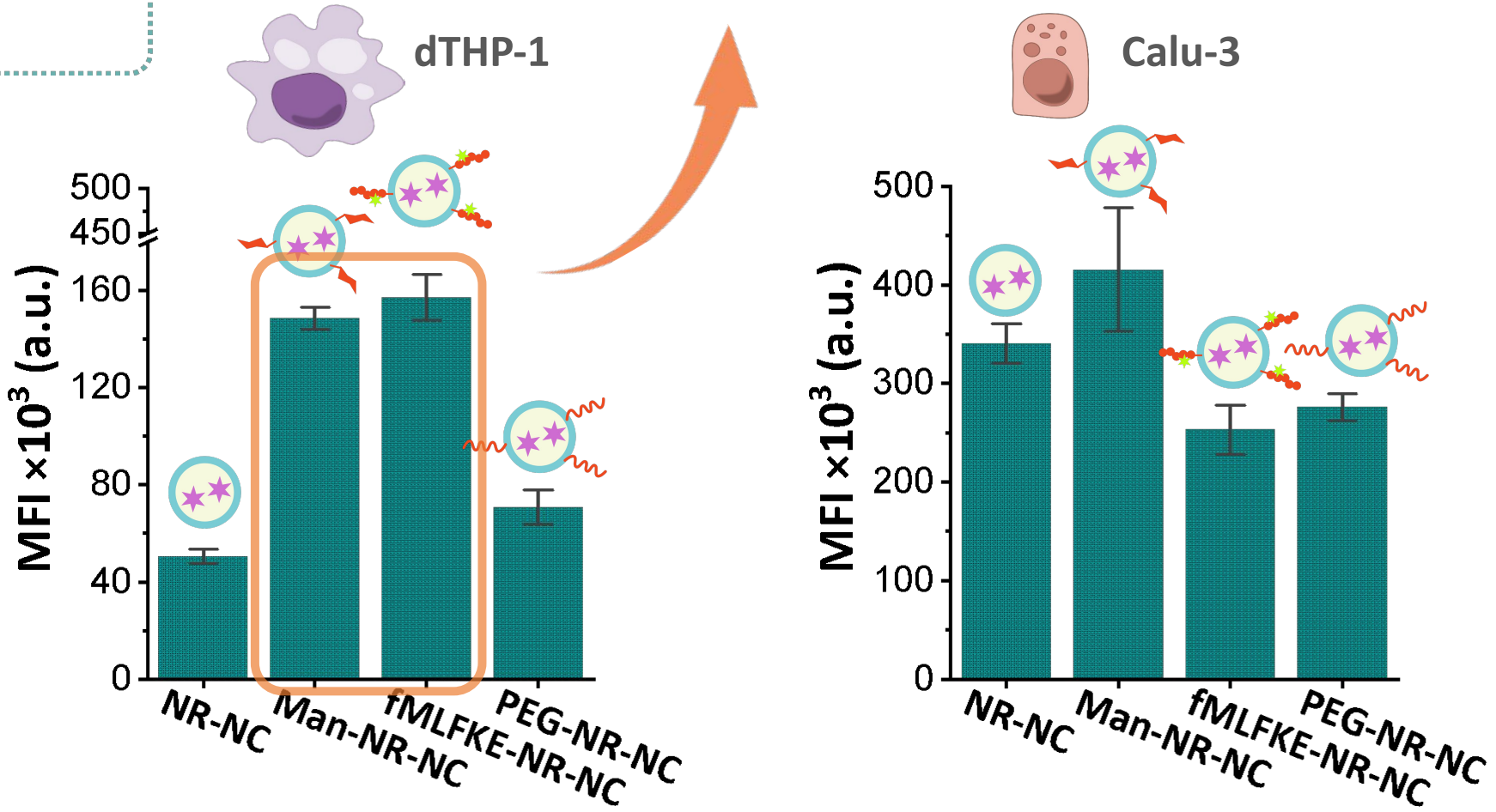
4. *In vitro* evaluation



▶ **Internalization**

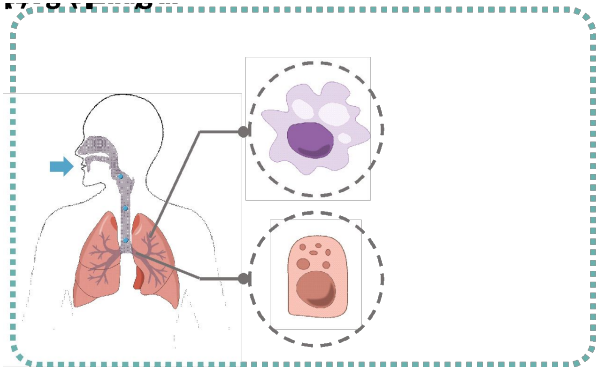
· Flow cytometry, 3-hour treatment, 0.3 mg/mL nanocapsules

Functionalized carriers: enhanced cellular uptake on macrophage model cells

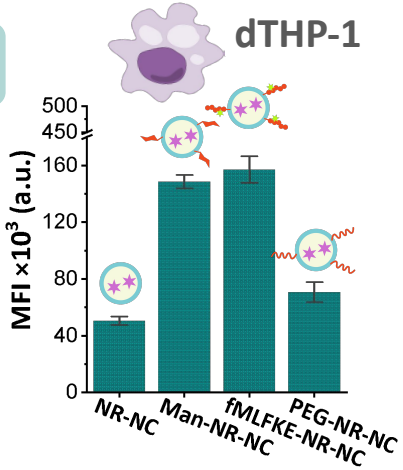


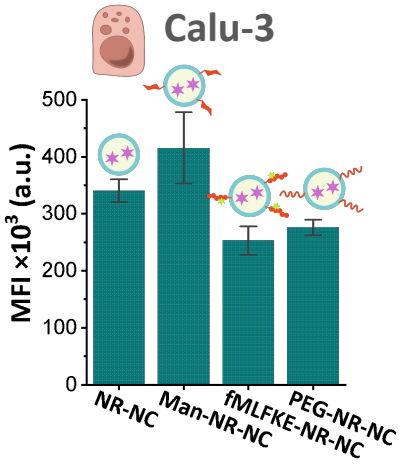
MFI: mean fluorescent intensity NR: Nile Red, excitation: 488 nm, detection: 640-740 nm (CytoFLEX)

4. *In vitro* evaluation



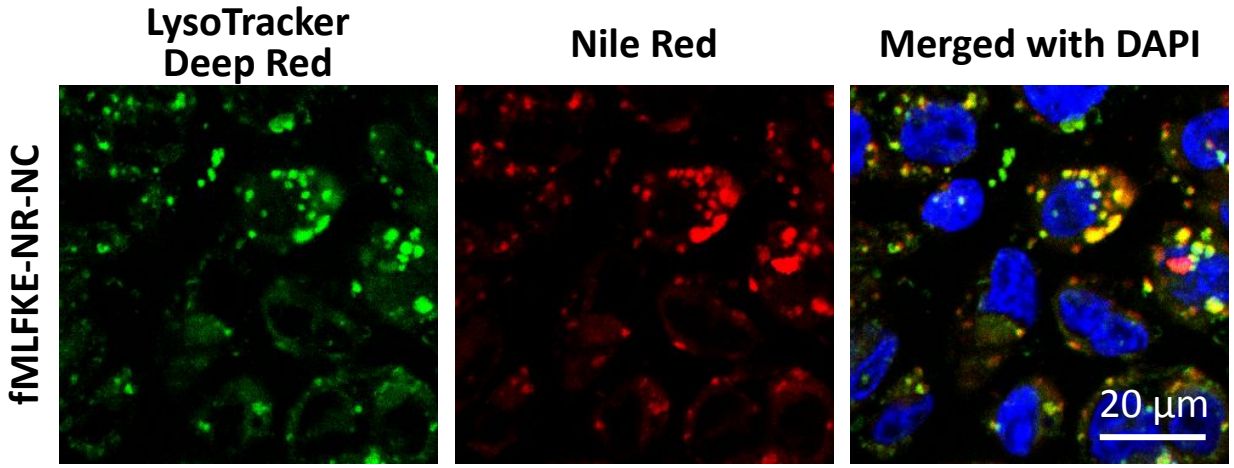
▶ Internalization





Functionalized carriers:
enhanced cellular uptake on macrophage model cells

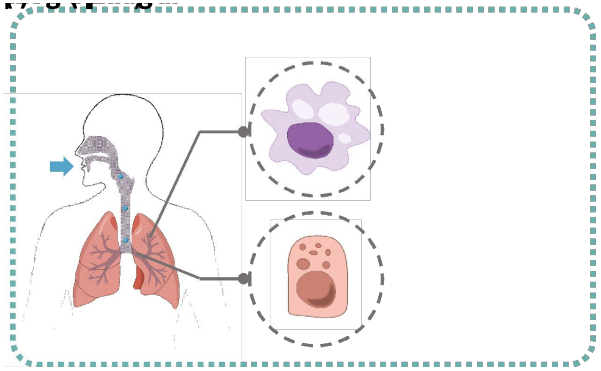
- Confocal microscopy, 3-hour treatment, 0.1 mg/mL nanocapsules



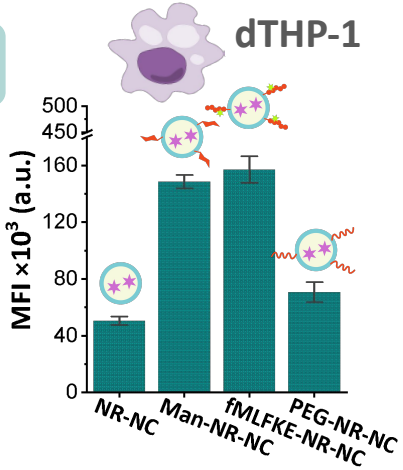
Nanocapsules: lysosomal localization

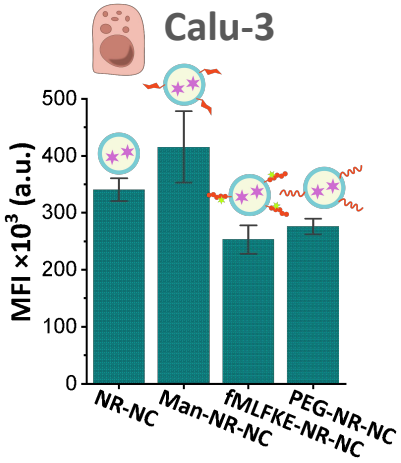
LysoTracker Deep Red: λex: 633 nm, λdet: 640-750 nm
Nile Red: λex: 488 nm, λdet: 535-635 nm
DAPI: λex: 405 nm, λdet: 410-510 nm
(Zeiss LSM 880)

4. *In vitro* evaluation



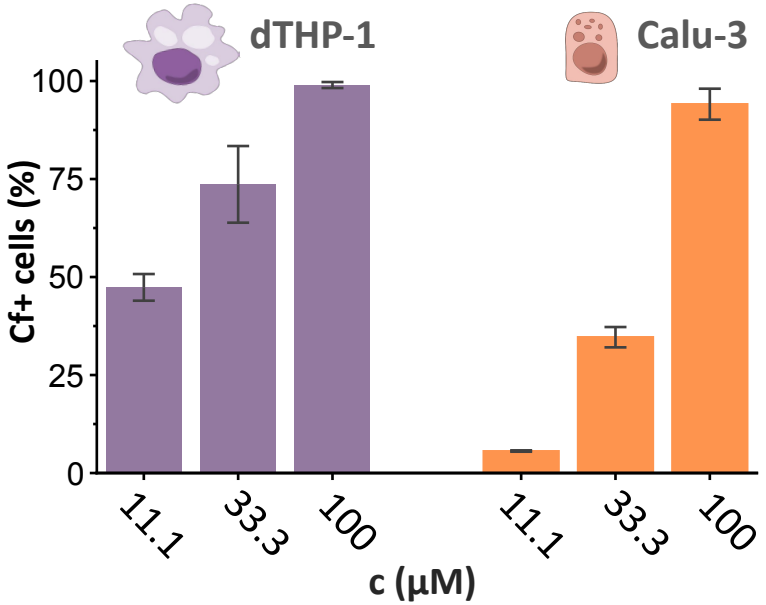
▶ **Internalization**

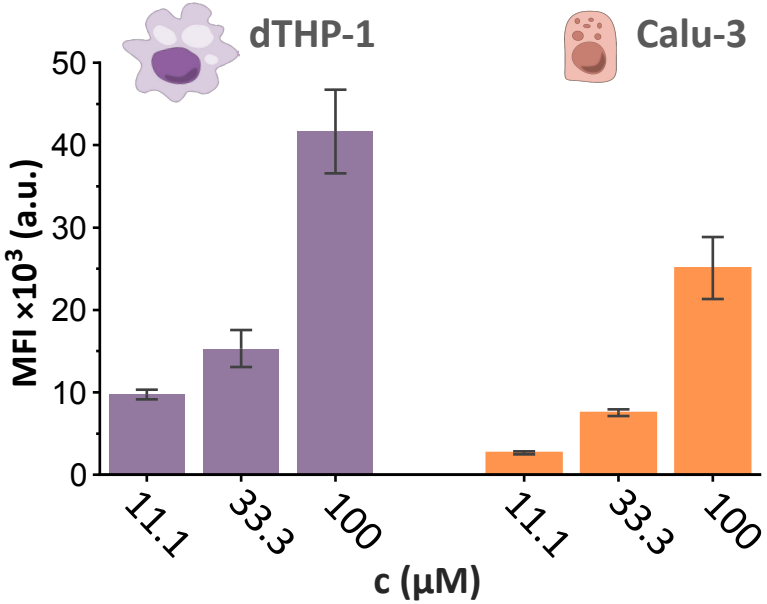


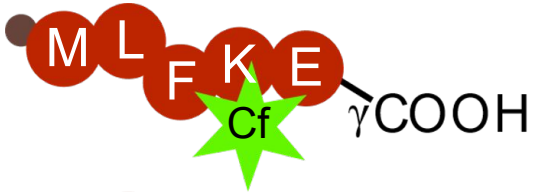


Functionalized carriers: enhanced cellular uptake on macrophage model cells

- Flow cytometry, 3-hour treatment with peptide





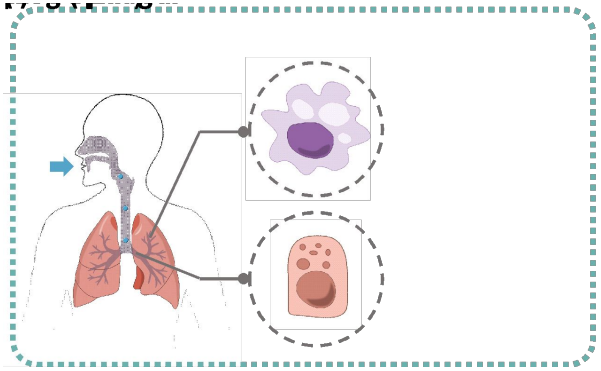


formyl-MLF peptide derivative

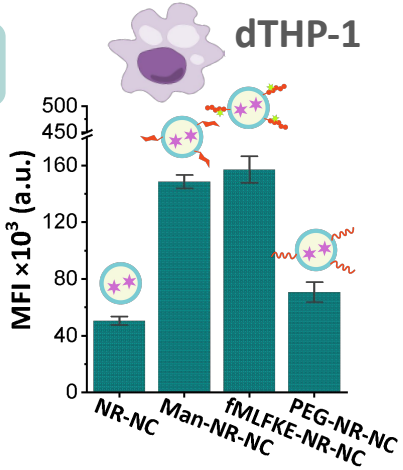
Higher peptide internalization in macrophages

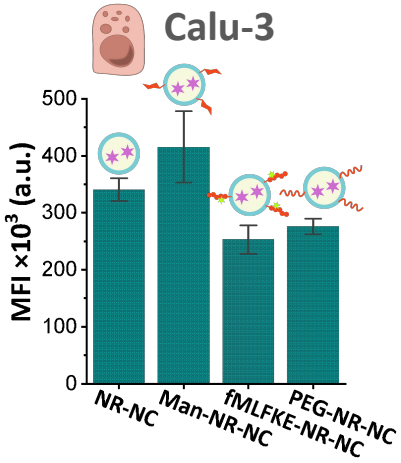
Cf: 5(6)-carboxyfluorescein, excitation: 488 nm, detection: 485-565 nm (CytoFLEX)

4. *In vitro* evaluation



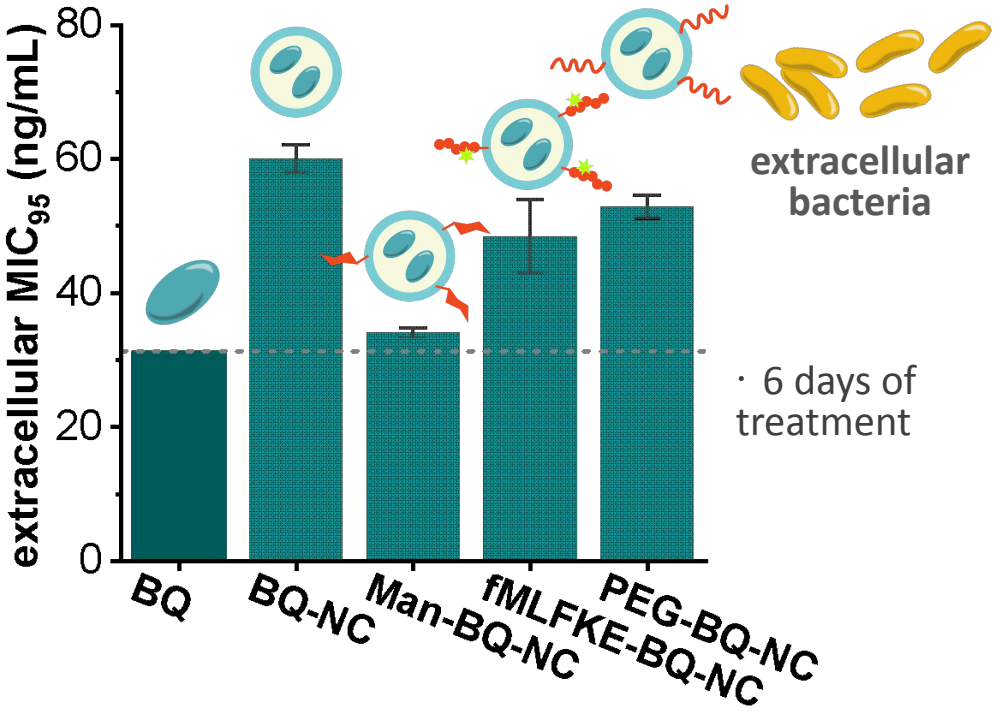
▶ Internalization

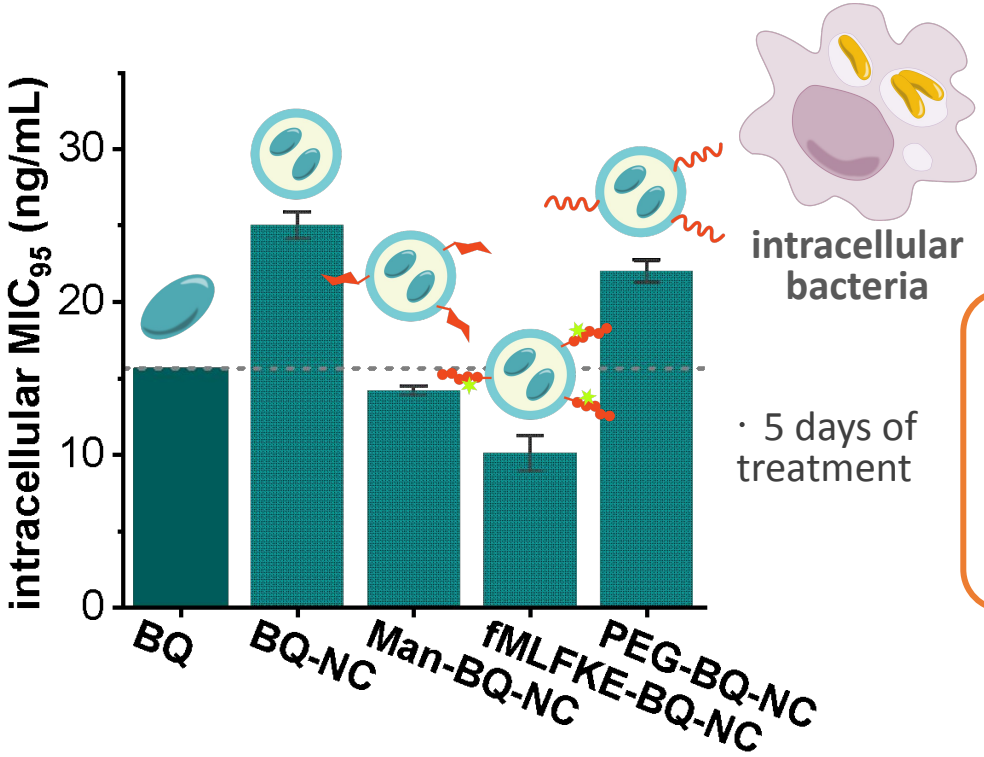




Functionalized carriers: enhanced cellular uptake on macrophage model cells

▶ Inhibition activity against *M. tuberculosis*

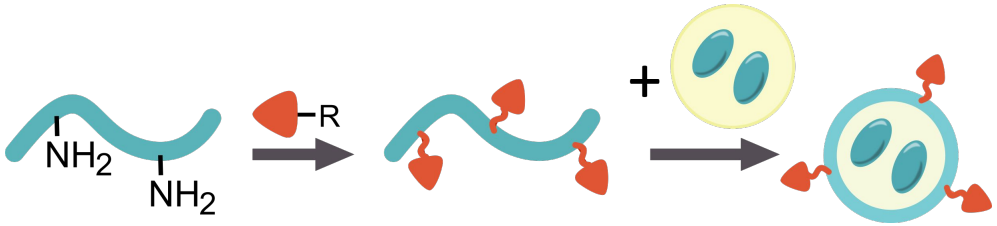


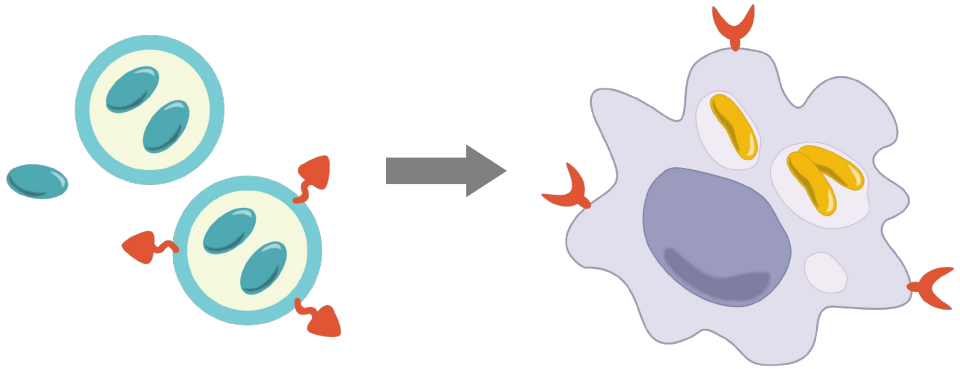


BQ-loaded carriers: maintaned activity of bedaquiline on extracellular and intracellular model

MIC: minimal inhibitory concentration, corresponding values to BQ-loading BQ: bedaquiline

5. Summary





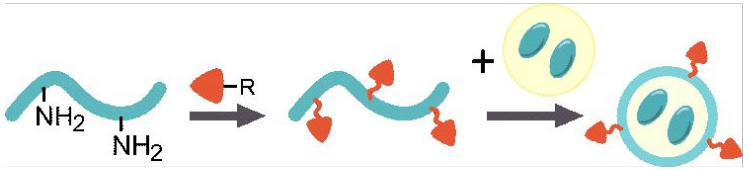
Preparation of NCs

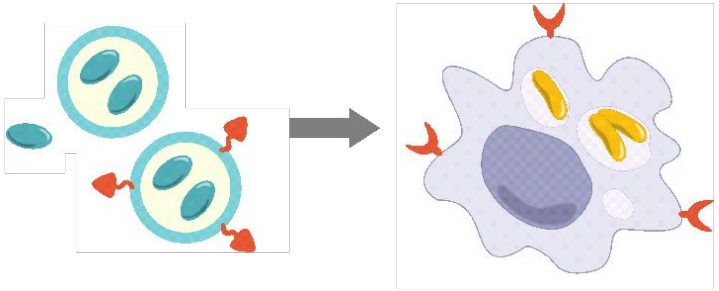
- Optimization of nanocapsule functionalization
- Efficient encapsulation of BQ into chitosan-based nanocapsules

Ligand functionalized NCs

- Enhanced cellular internalization
- Outstanding inhibition of extra- and intracellular bacteria

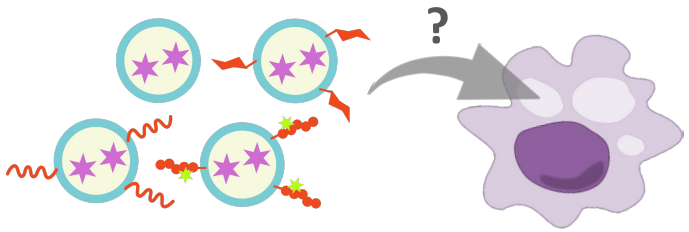
5. Summary



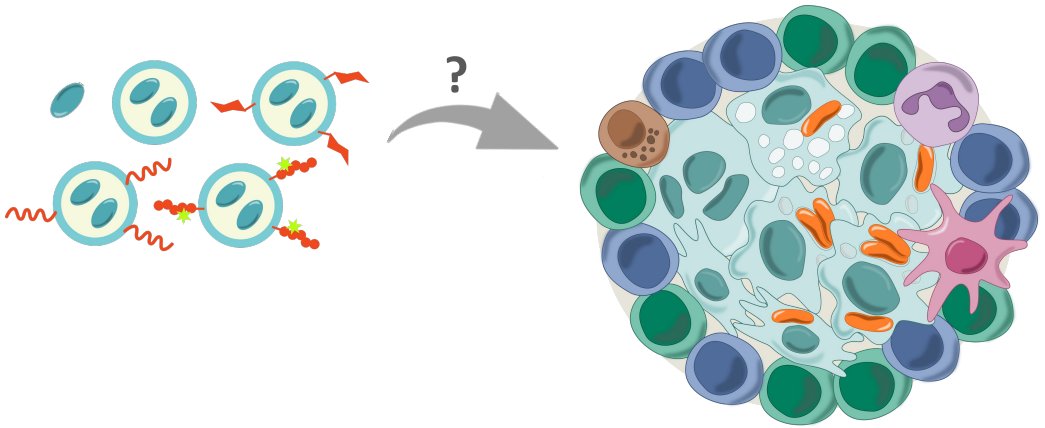


6. Future plans

► internalization mechanism studies



► studies on *in vitro* granuloma model



Acknowledgement

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Thank you for your attention!

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