

Cationic complexes in Transfection

Cationic complexes consist of DNA bound by a cationic polymer
These polymers contain positive/cationic functional groups
These are common agents in transfection
Where genetic material is delivered into cells

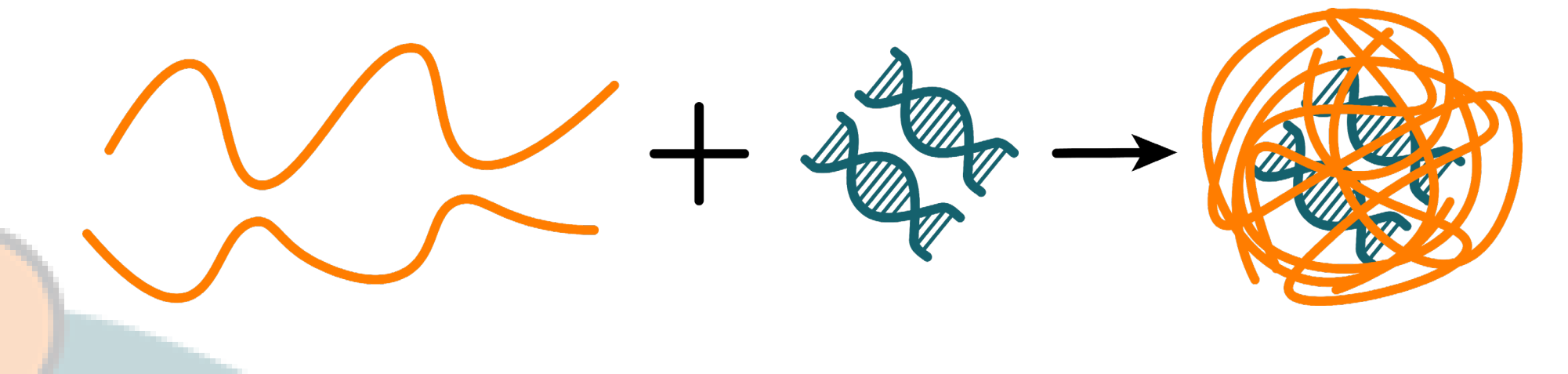
Cationic systems are advantageous due to their¹:

- Ease of preparation
- Modifiability
- Higher cargo capacity

Polymer systems utilize the binding of it’s positive functional groups with the negatively charged phosphates in the DNA backbone to form a polyplex^{2,3}

These systems work by mask the anionic charges of the DNA allowing for the polyplex to bind to the cell membrane triggering endocytosis^{4,6}

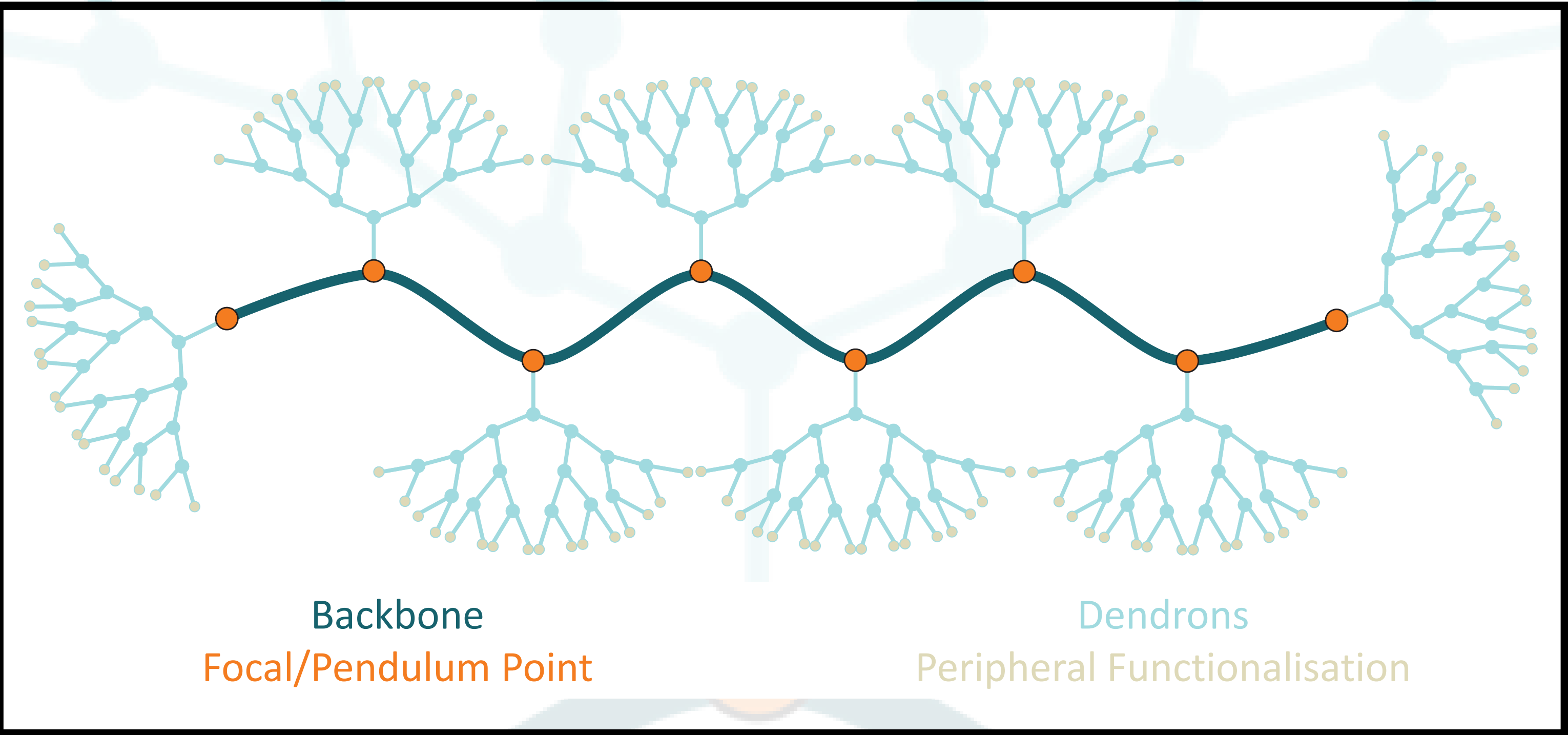
During endocytosis the polymer independently or with the aid of a secondary agent causes endosomal escape releasing the DNA into the cell⁵⁻⁷



Hacking the mechanisms of a cell:

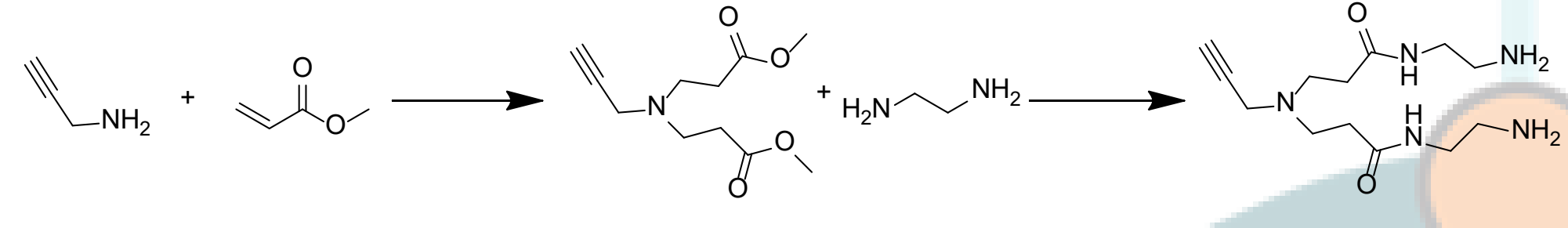
Developing a Trojan system based on a sequence defined dendritic polymer system to deliver genetic material

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PAMAM Dendrons

- ❖ Poly(amidoamine) dendrons consist of repeating units of amide and amine functional groups
- ❖ Each repeating polymerisation creates a two directional split creating a branching pattern
- ❖ PAMAM is synthesised through repeated reactions between methyl acrylate and ethylene diamine
- ❖ PAMAM dendrons contain both primary and tertiary amines⁵
 - ❖ The primary amines will predominantly bind DNA
 - ❖ The tertiary amines predominantly provide the necessary buffering capacity for endosomal escape



Cationic polyplex infiltration and expression

Infiltration begins with the attachment of the polyplex to the cell membrane

Stage 1: Bypassing the membrane

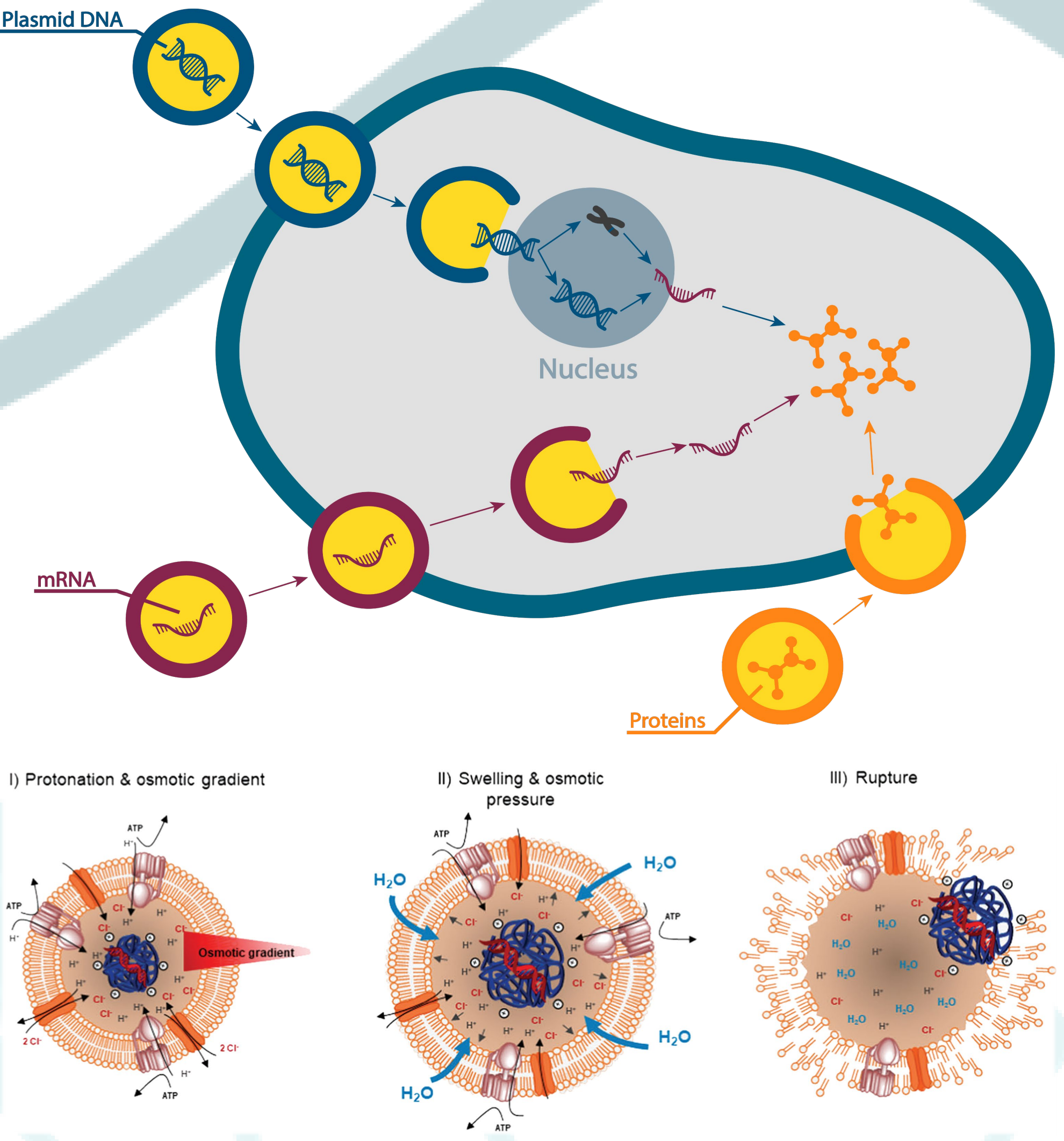
1. Polyplex attaches to the cell membrane to trigger endocytosis
2. Polyplex is internalised within a membrane-bound vesicle, moving into the cytosol

Stage 2: Breaking out^{6,7}

1. The early endosome within which the polyplex is contained starts down the lysosomal pathway
2. This starts the acidification of the endosome where the V-ATPase moves H⁺ into the endosome, and for ionic balance Cl⁻ is introduced through chloride channels
3. The polyplex absorbs the H⁺ acting as a buffer and keeping the pH neutral
4. This lack of change in pH encourages more H⁺ ion influx resulting in a build-up of osmotic pressure with increasing Cl⁻ ions
5. This pressure causes an influx of water, which strains the endosomal membrane to critical membrane failure, releasing the polyplex into the cytoplasm

Stage 3: Planting Data

- ❖ For RNA and proteins, release into the cytosol is sufficient to induce expression within the cell¹.
- ❖ For DNA to be expressed it must be within or close to the nucleus. To achieve deliver DNA into the nucleus biological vectors (viruses) can hijack the dynein and kinesin motor proteins to deliver the DNA to the nucleus directly^{8,9}



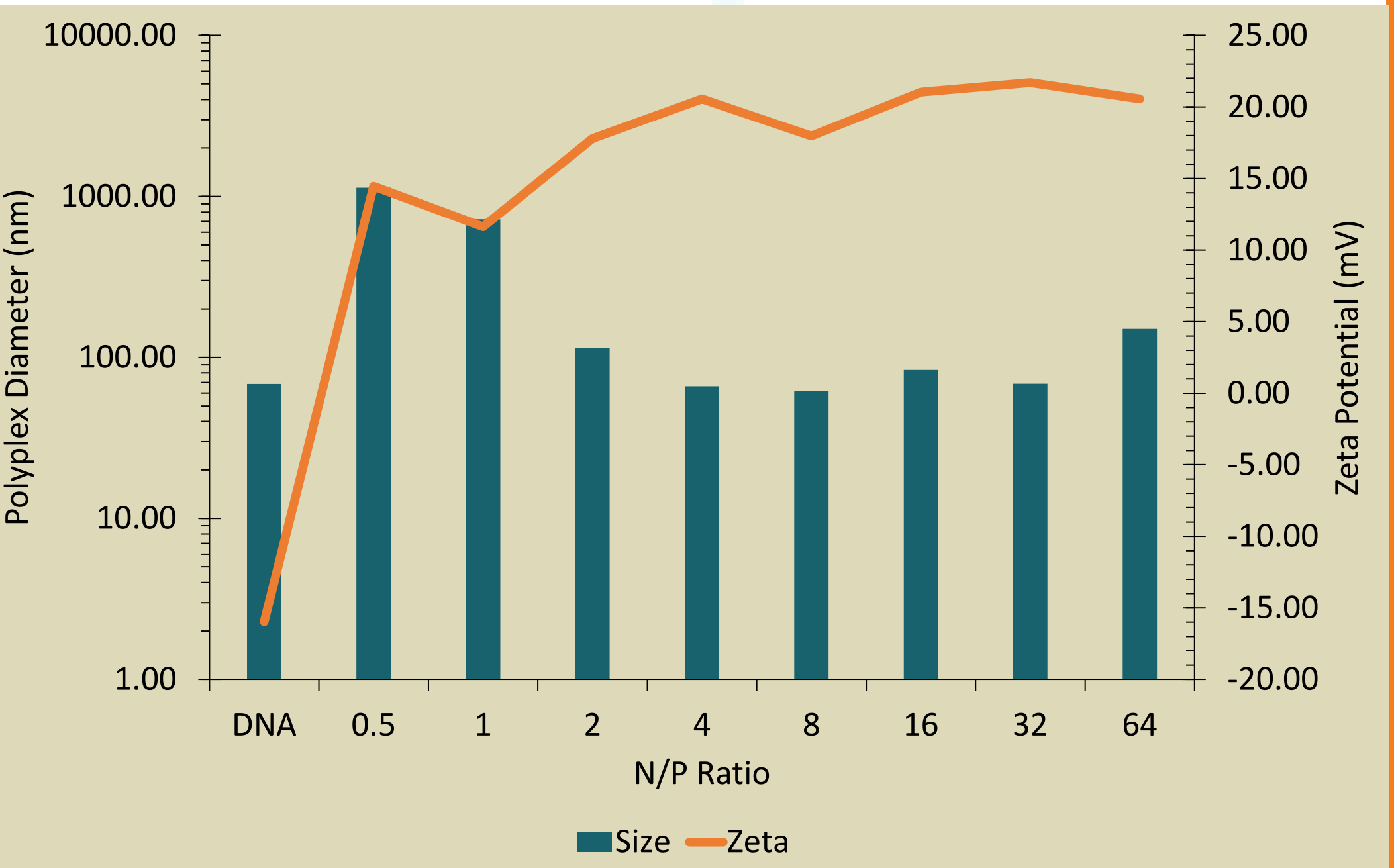
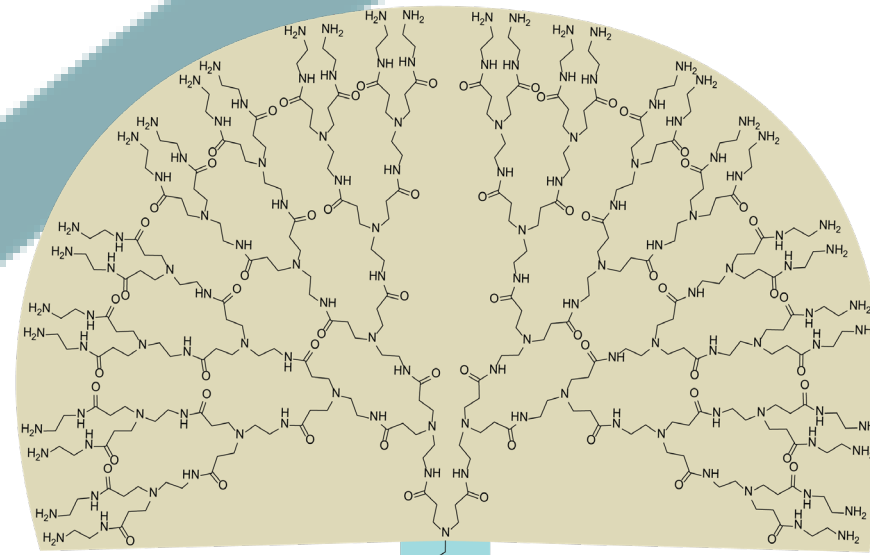
Prototype polymer analysis

The initial tests have been completed on a prototype polymer (2n D3)

- ✓ DLS size measurements
- ✓ Zeta potential measurements

Further analysis will be made in:

- ☐ DLS (size and zeta potential) at various N/P (Amine/Phosphate)
- ☐ DNA binding Assay
 - ☐ Gel
 - ☐ PicoGreen assay
- ☐ Transfection:
 - ☐ Single 5kb Plasmid
 - ☐ Two 5kb Plasmid
 - ☐ 5kb and 10kb Plasmid



Polymerisation of components

Current prototype system

The synthesised prototype backbone is done using two reactions:

DCC DMAP

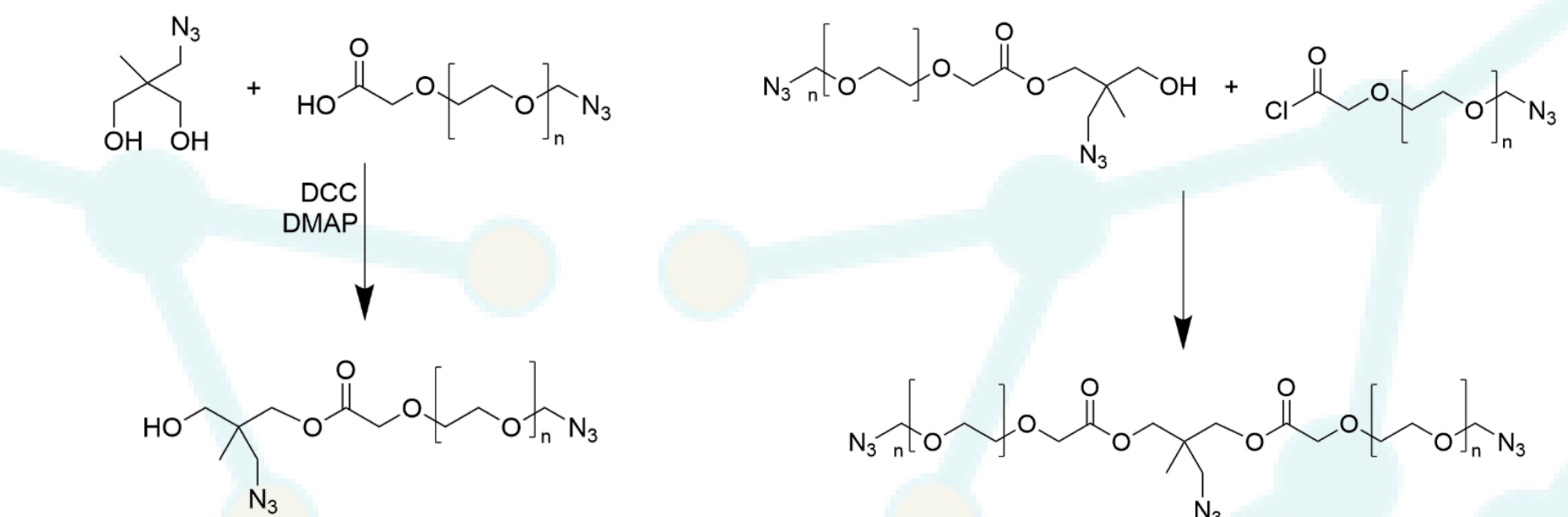
- ❖ One sided addition of chain or cap to linker
- ❖ Limited reaction through steric effects

Acid Chloride

- ❖ Used to extend or cap chain
- ❖ Reacts with free alcohol on junction after DCC reaction

Method works for prototyping

Improved methods would be important for future work and streamlining synthesis



Modular components for assembly

Modular assembly allows for new synthetic polymers to be generated more easily and faster

The system devised for this project utilises:

- ❖ Space out functional groups
- ❖ Act as a biocompatible backbone
- ❖ Azide focal sites:
- ❖ Bind dendrons through click reactions

Various functional groups to polymerise the backbone

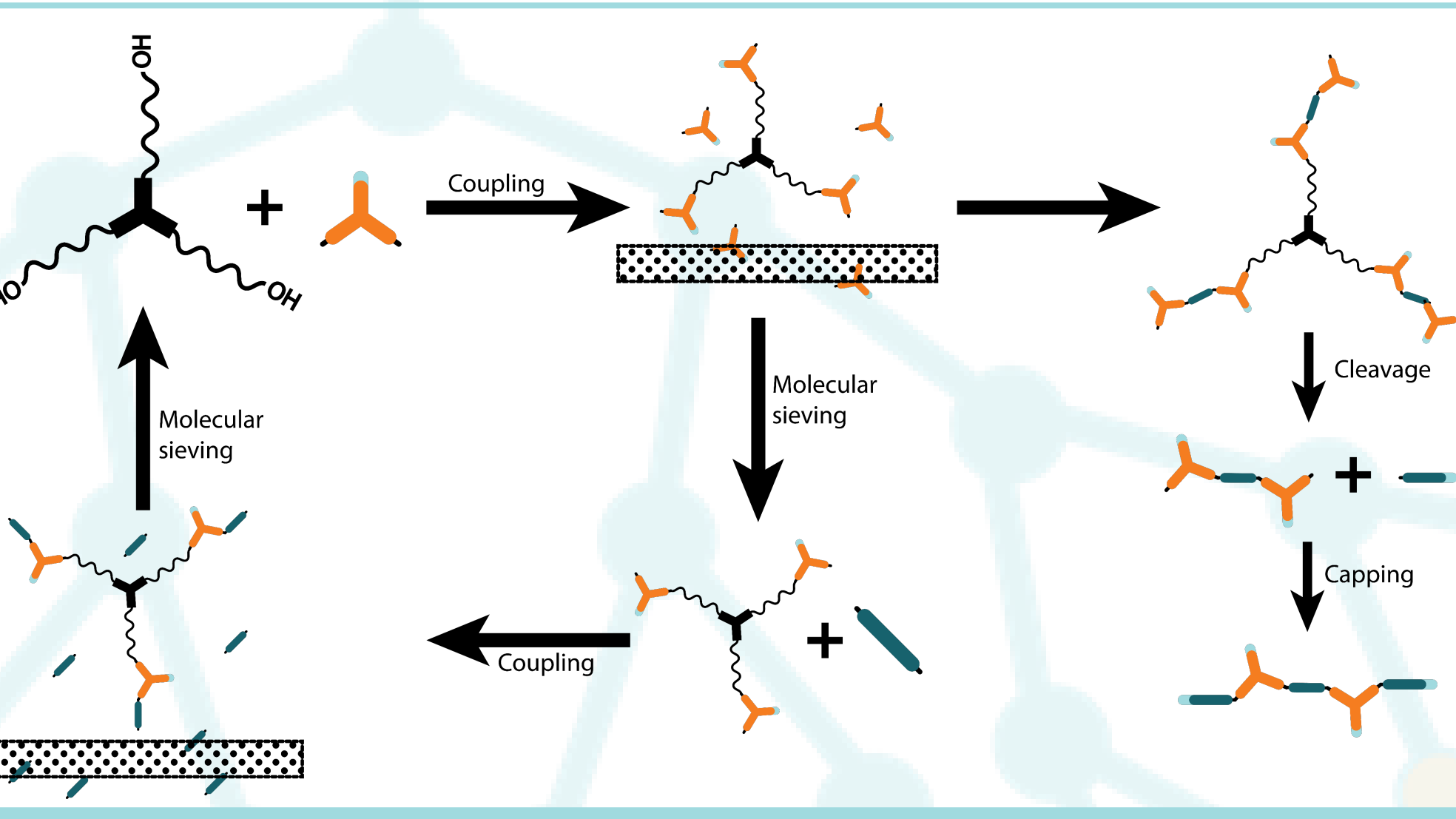
- ❖ Alcohol
- ❖ Carboxylic acid
- ❖ Amine

Junction	Chain	Capping chain
Three-way junction between components	Longer monomer that spans between junction monomers	Long monomer chain that ends or starts the polymer chain

Liquid phase cyclic polymerisation

Adapting the cyclic procedure from Dong *et al.*¹⁰

- ❖ Improved control over polymer length and composition
- ❖ Cyclic process allows for faster synthesis
- ❖ Adaptable to new polymer binding systems (amide/peptide)



References

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