School of Chemical Engineering

School of Biotechnology and Biomolecular Sciences

# Antifungal Polymer Design:
## *Impact of Defined Block-sequence on Antimycotic and Hemolytic Activities*

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38APS
38TH AUSTRALASIAN POLYMER SYMPOSIUM
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# *Fungal Infections*

Picture from podcasts: Spotify.com and VOX.com

**what about fungal infections ?**

# *Invasive Fungal Infections and Clinical Challenges*

**Fungal disease incidence and mortality**

**3,752,000 death**
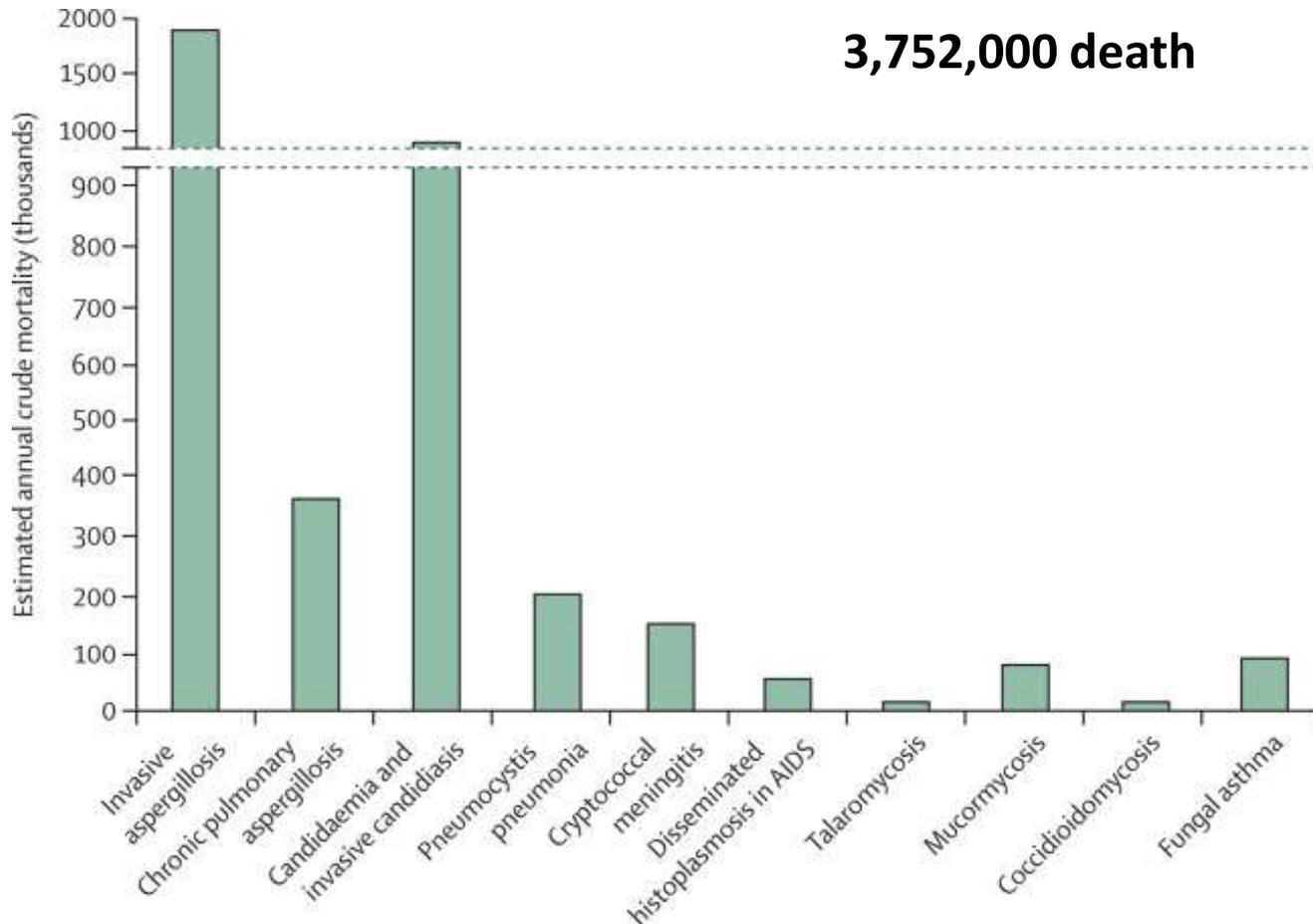


**Current Issues**

*Emerging fungal infections

*Drug resistance

*Limited therapeutic options

*Drug development challenges

Denning, D.W., *The Lancet Infectious Diseases*. (2024)
Perfect, J.R., *Nature reviews Drug discovery*, (2017 *16*(9), pp.603-616.

# Bio-mimic Antimicrobial Peptides

**Histatin-5:  human salvia peptide with 24 amino acids**
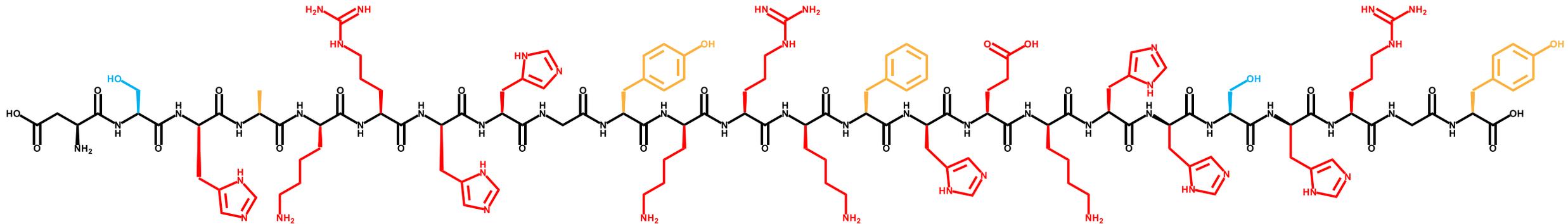


**Histatin-5: Peptide Limitation**
- **Metabolic instability: susceptibility to proteases**
- **Antifungal activity: reduced *in vivo***

- Overcome the issue with bioactivity and toxicity
-  Increase production scale
- Reduce cost of synthesis
- Modulate the physicochemical properties
- Improve therapeutic performance

# *Nanoengineered Synthetic Antifungal Polymers*

**Synthetic Monomers**
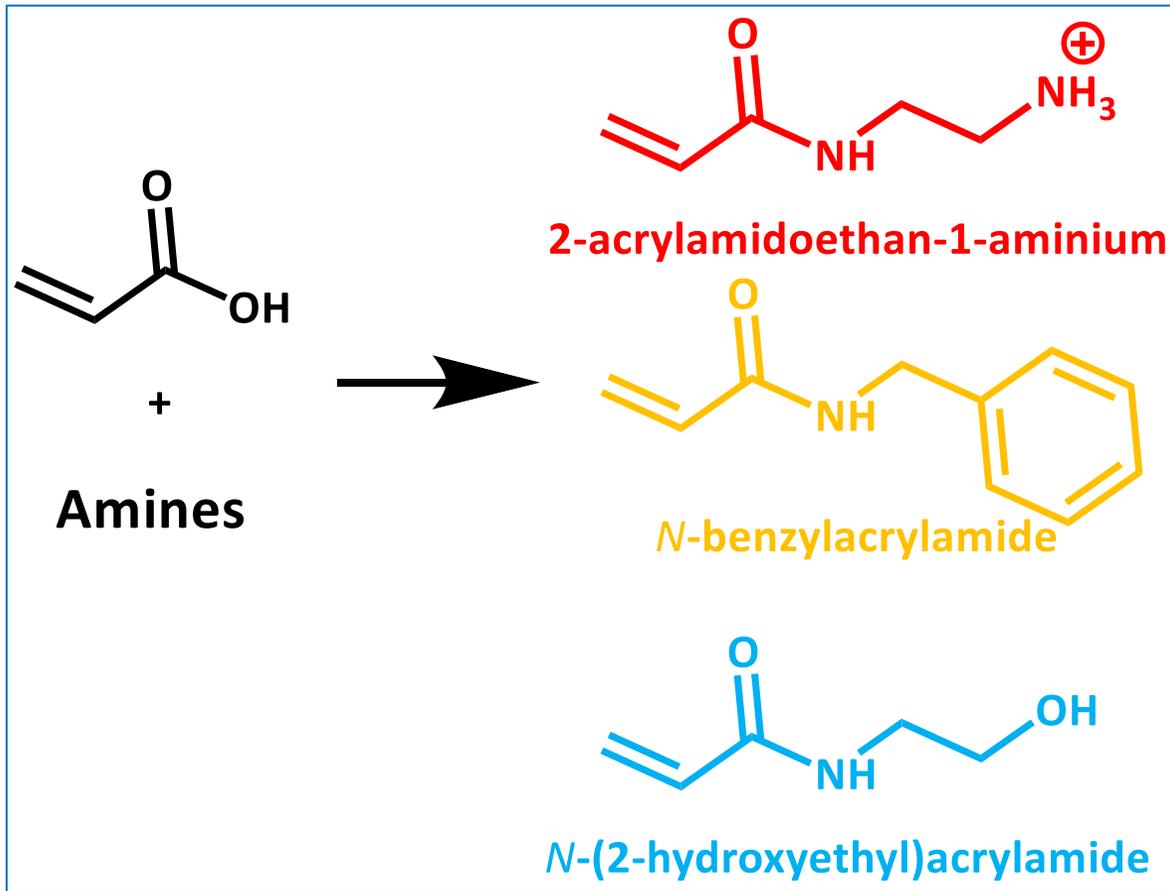
**High-throughput Synthesis**



PET-RAFT
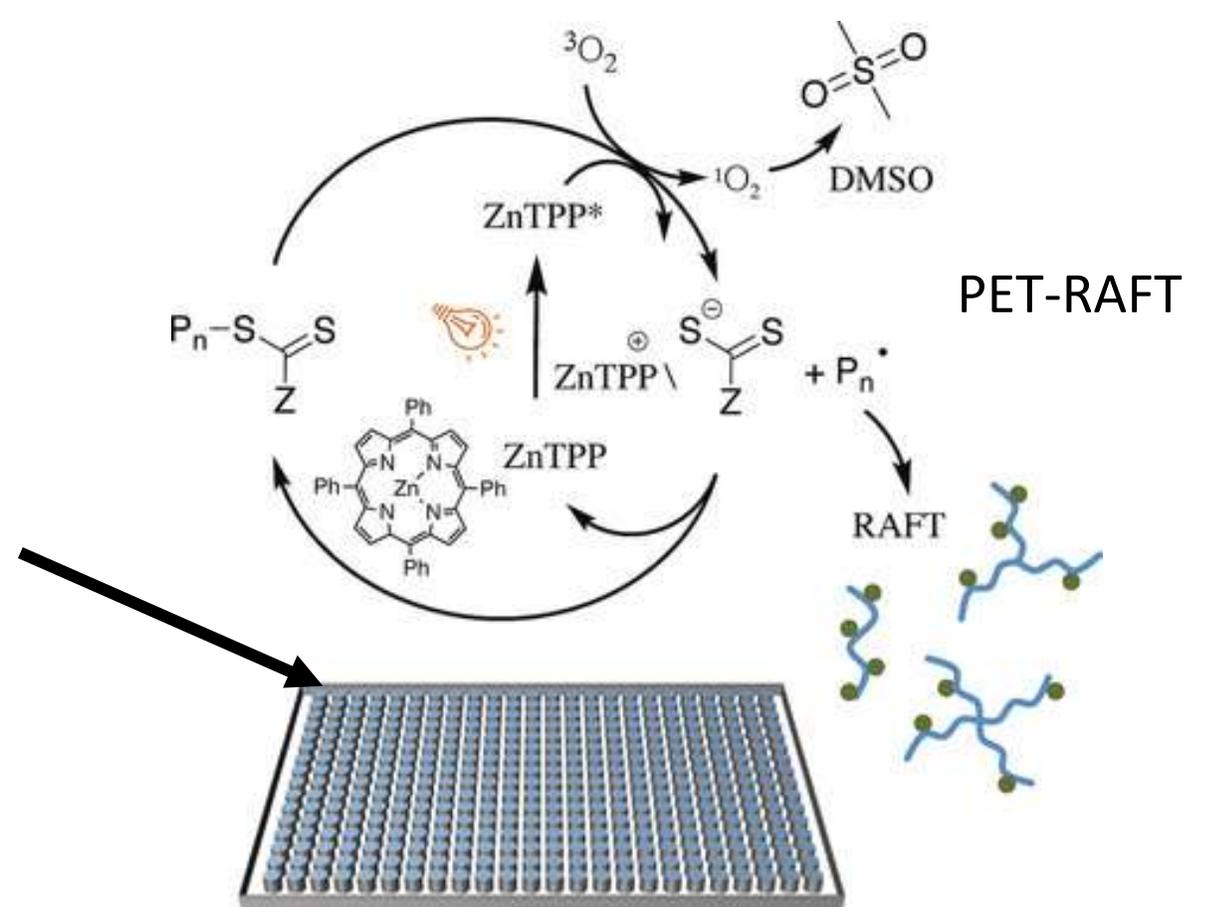
# Rational Design of Statistical Linear Antifungal Polymer



Primary Ammonium

Guanidinium

Imidazole

Carboxylic acid

Ratio Composition, (50/25/25)
Degree of Polymerization (20 $X_n$)

N-isopentylacrylamide

N-propylbutylacrylamide

N-pentylacrylamide

N-(cycloheptyl)acrylamide

N-heptylacrylamide

N-(cyclohexylmethyl)acrylamide

Schaefer, S., Pham, T.T.P., Brunke, S., Hube, B., Jung, K., Lenardon, M.D. and Boyer, C.,.*ACS Applied Materials & Interfaces*, (2022) 13 (23), pp.27430-27444.
Schaefer, S., Melodia, D., Pracey, C., Corrigan, N., Lenardon, M.D. and Boyer, C.,. Biomacromolecules. (2022 ).

# *What is the Effect of the Controlled Block-sequence?*



A 🔴 Cationic

B 🟡 Hydrophobic

C 🔵 Hydrophilic

**Random**
**20** units: **50%** cationic, **25 %** hydrophobic and **25%** hydrophilic

Di-Block

Tri-Block
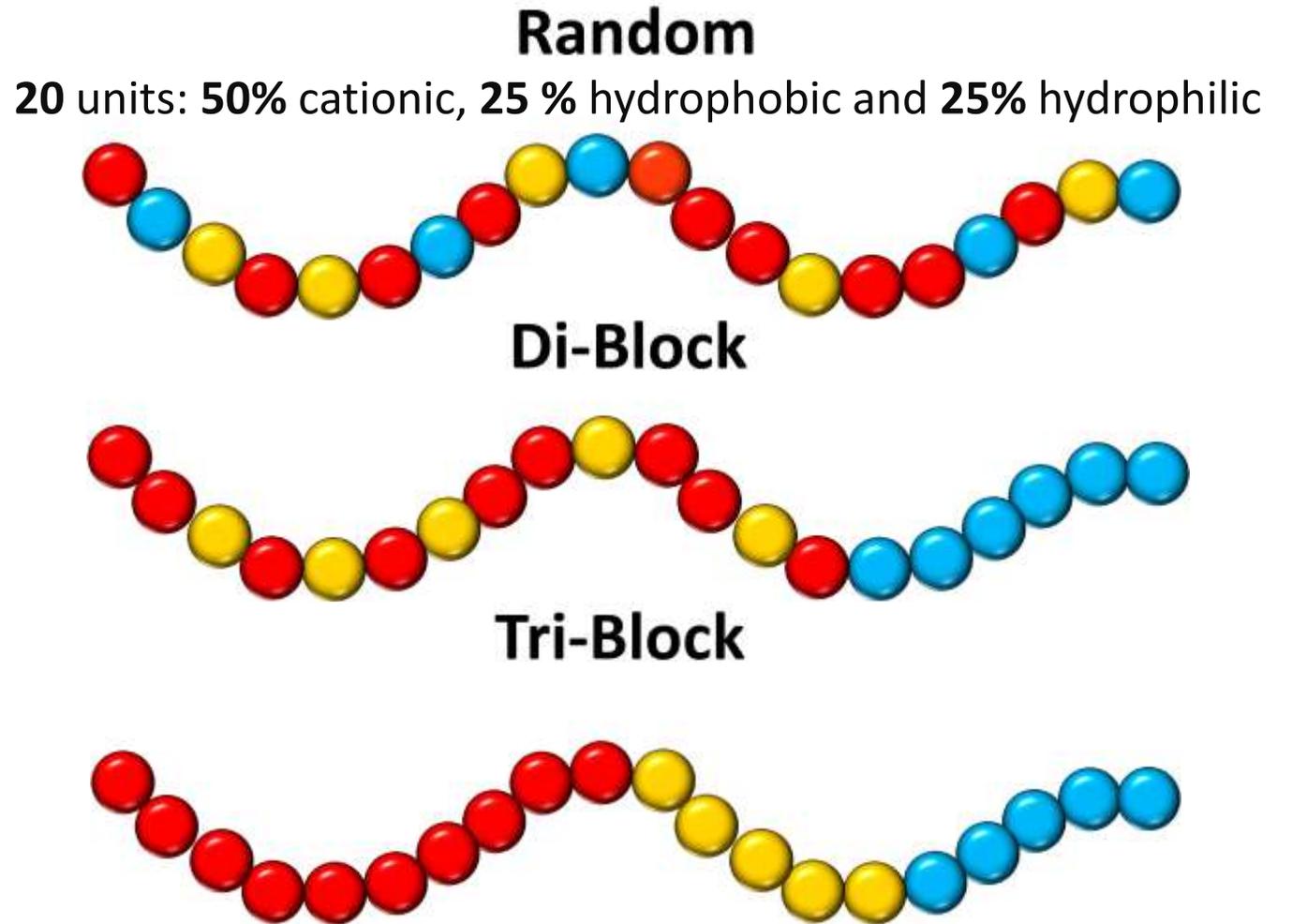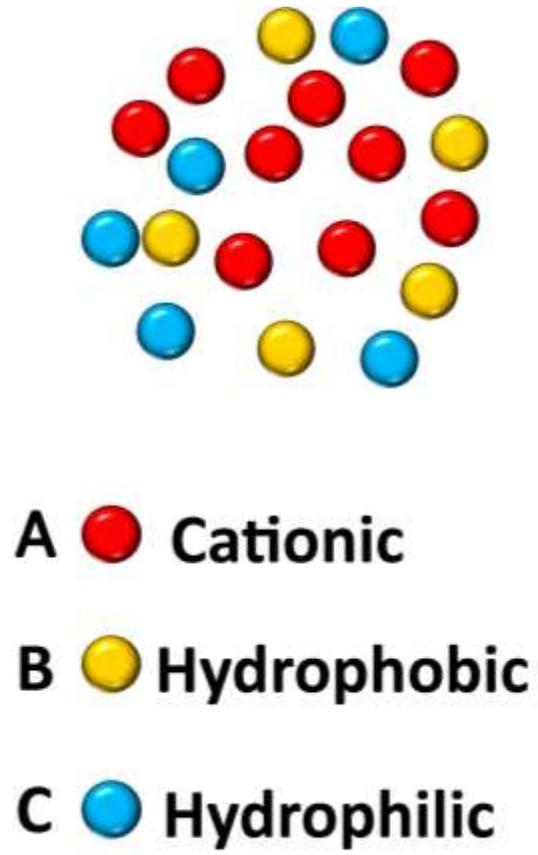
# Research Overview
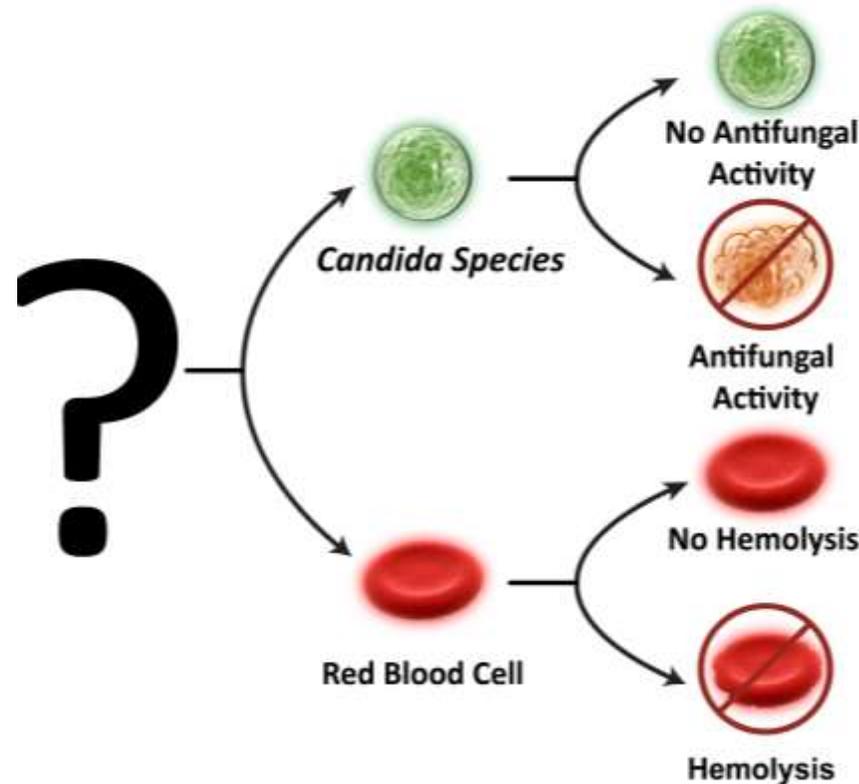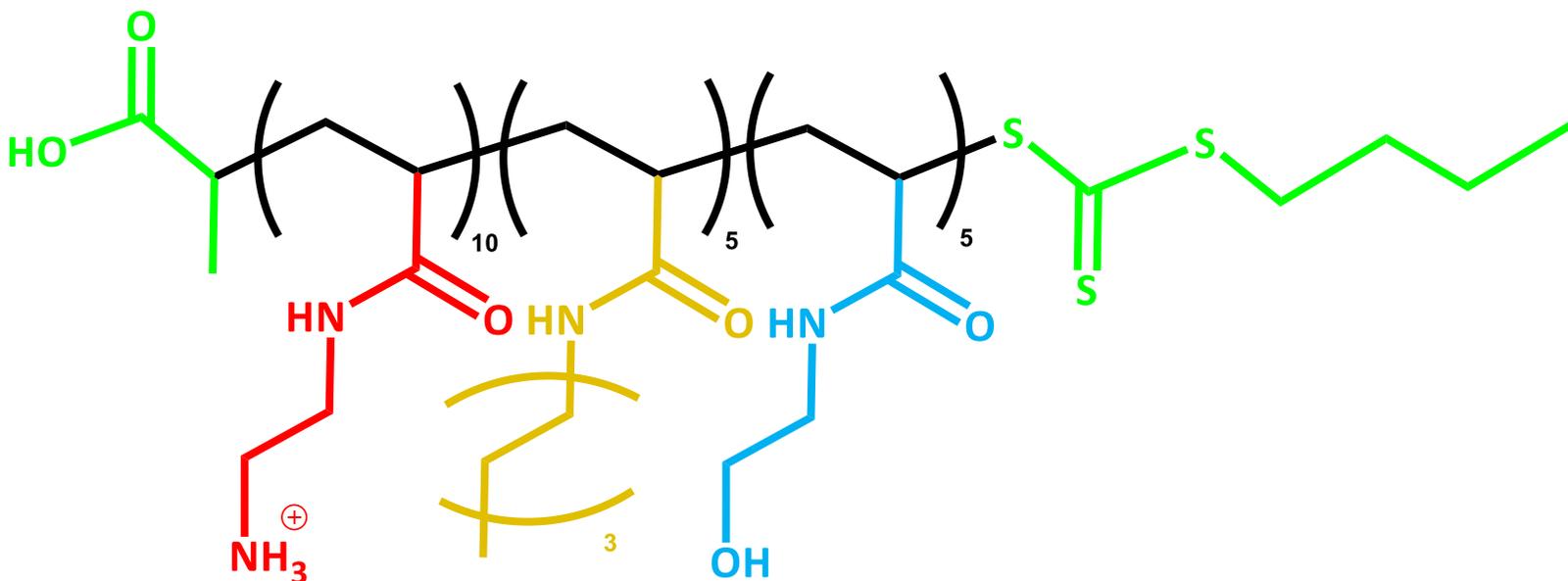


**A-Cationic 50%:** 2-acrylamidoethan-1-aminium)

 **B: HydrophobiC 25%:** *N*-heptyl acrylamide

**C: Hydrophilic 25%:** *N*- (2-hydroxyethyl) acrylamide
)

2-(butylthiocarbonothioylthio) propanoic acid (BTPA)
**Initial component:** Isobutyric acid (R group)
**End group:** Living thiol group (Z group)

# Defined Multi-block Polymer Design



*tert*-butyl (2-acrylamidoethyl) carbamate (A)

*N*-heptylacrylamide (B)

*N*-(2-hydroxyethyl) acrylamide (C)

PET-RAFT Polymerization

Defined Ternary Block A-B-C
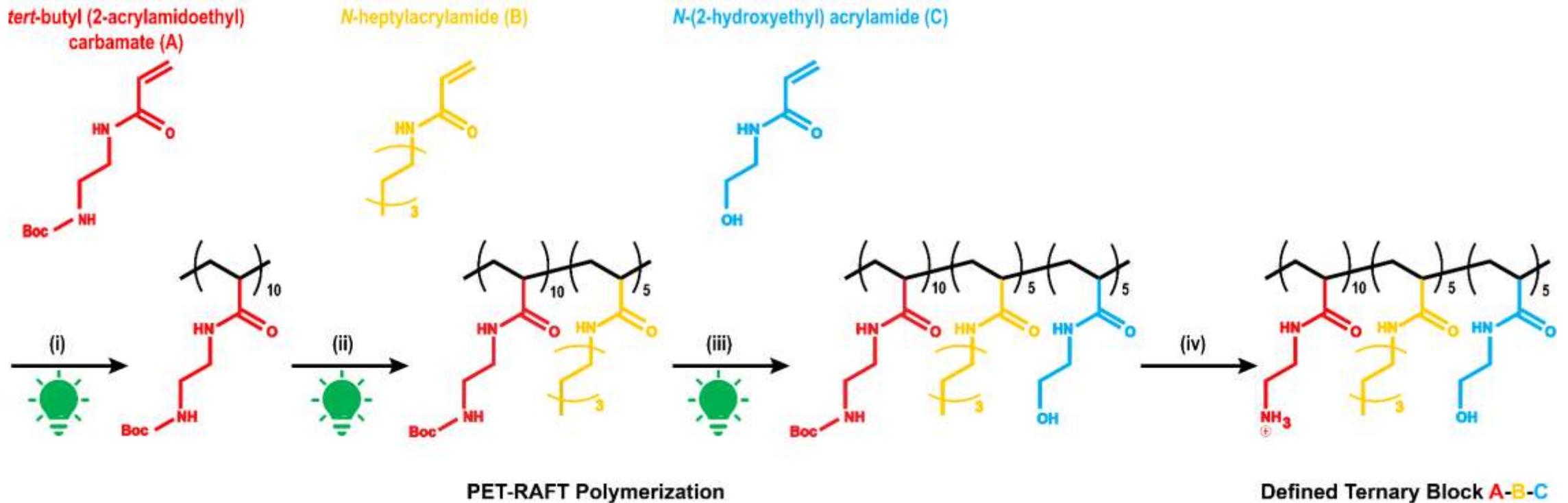
- In one-pot synthesis
- 25 % w/w in DMSO
- 20 h under green light source (λ=530 nm, Io = 5.8 mW/cm$^2$)

- 100 ppm of Zinc tetraphenylporphyrin (ZnTPP) to monomers
- BTPA): RAFT Agent
- Direct Boc-deprotection- TFA

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# *Characterization of Defined Multi-block Polymers*
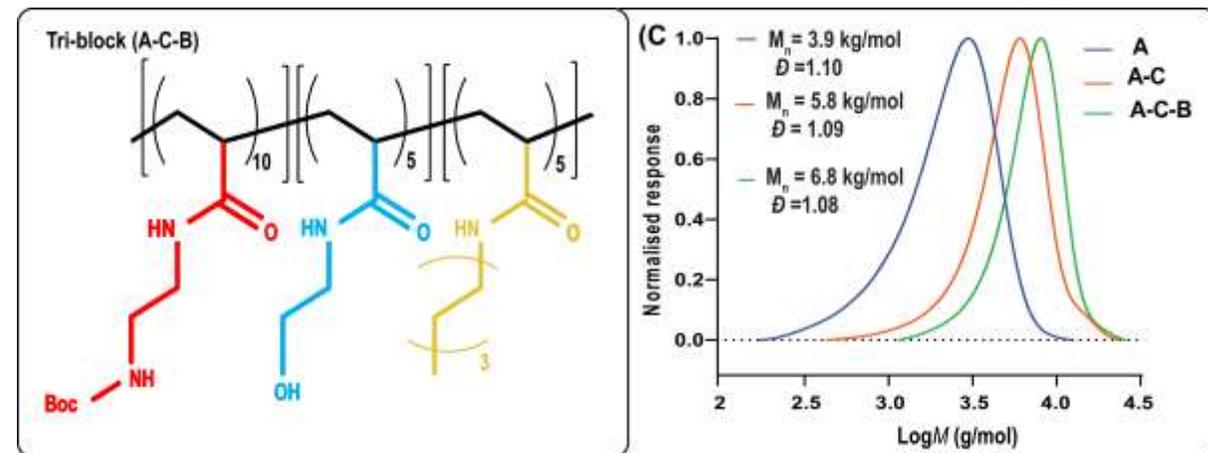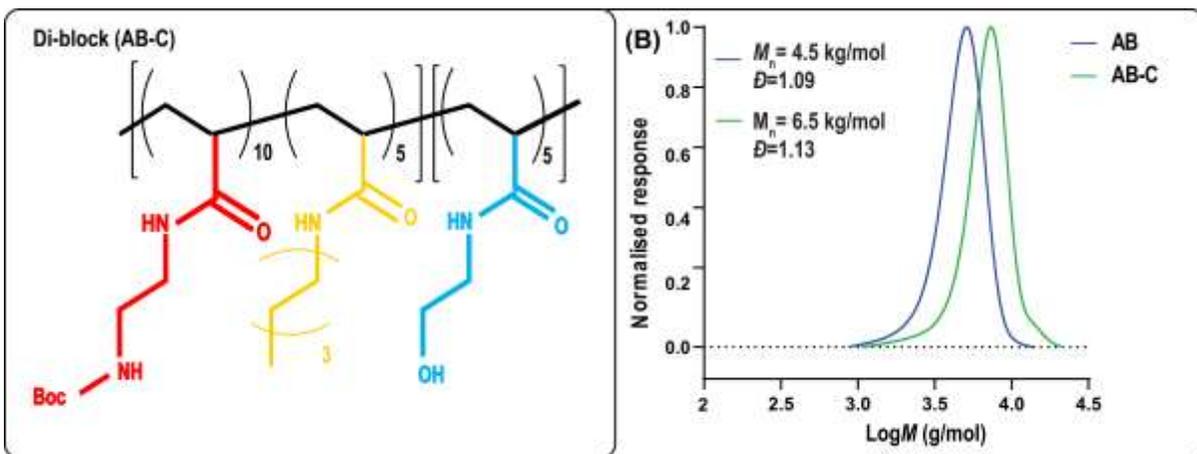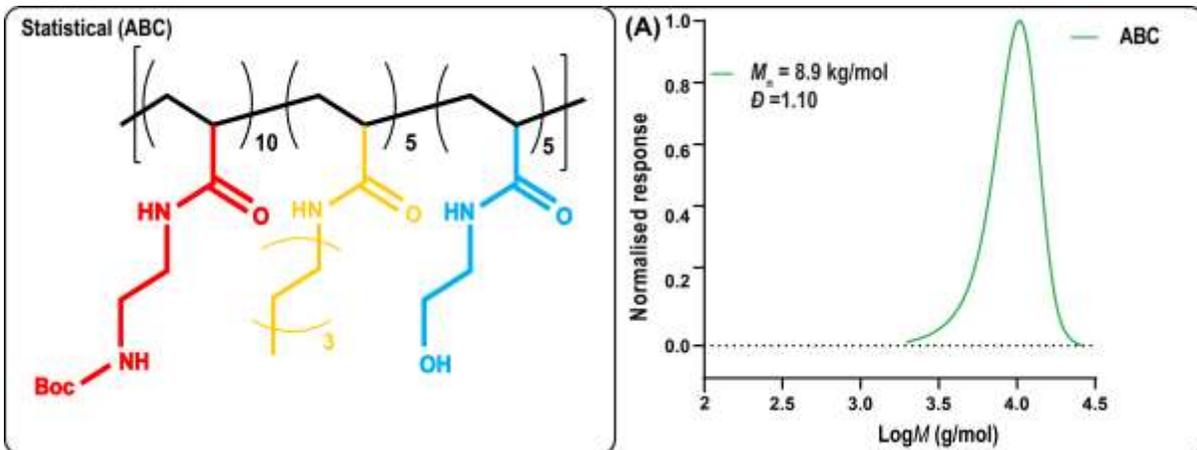


- **Controlled polymerization**
- **Increasing molecular weight in each block addition**

# Characterization of Defined Multi-block Polymers

| Ref. | Sequence A, B, C | Structure | Target $X_n$ | Target ratio A:B:C | Target $M_n$ [a] (g/mol) | $X_n$ NMR [b] | $M_n$ [c] (g/mol) | Composition [d] A: B: C | $M_n$ [e] (g/mol) | $Đ$ [f] |
|---|---|---|---|---|---|---|---|---|---|---|
| R-01 | ABC | Statistical | 20 | 50:25:25 | 3800 | 20 | 3800 | 50:24:26 | 8900 | 1.10 |
| B-01 | A-B-C | Tri- block | 20 | 50:25:25 | 3800 | 20 | 3780 | 49:26:25 | 6800 | 1.12 |
| B-02 | C-B-A | Di-block | 20 | 50:25:25 | 3800 | 21 | 3970 | 48:28:24 | 6600 | 1.13 |
| B-03 | A-C-B | Di-block | 20 | 50:25:25 | 3800 | 20 | 3840 | 51:27:22 | 6350 | 1.10 |
| B-04 | B-C-A | Di-block | 20 | 50:25:25 | 3800 | 20 | 3850 | 54:22:24 | 6800 | 1.11 |
| B-05 | B-A-C | Tri- block | 20 | 50:25:25 | 3800 | 20 | 3810 | 50:26:24 | 6800 | 1.08 |
| B-06 | C-A-B | Tri- block | 20 | 50:25:25 | 3800 | 20 | 3860 | 52:26:22 | 8900 | 1.11 |
| B-07 | AB-C | Tri- block | 20 | 50:25:25 | 3800 | 20 | 3690 | 54:24:22 | 8650 | 1.09 |
| B-08 | C-AB | Tri- block | 20 | 50:25:25 | 3800 | 20 | 3780 | 48:28:25 | 5700 | 1.12 |
| B-09 | A-BC | Tri- block | 20 | 50:25:25 | 3800 | 21 | 4040 | 52:26:22 | 6700 | 1.11 |
| B-10 | BC-A | Di-block | 20 | 50:25:25 | 3800 | 18 | 3460 | 53:21:26 | 4500 | 1.16 |
| B-11 | AC-B | Di-block | 20 | 50:25:25 | 3800 | 20 | 3790 | 49:26:25 | 5250 | 1.14 |
| B-12 | B-AC | Di-block | 20 | 50:25:25 | 3800 | 20 | 3790 | 49:26:25 | 5250 | 1.14 |

A: *tert*-butyl (2-acrylamidoethyl) carbamate, B: *N*-heptyl acrylamide, C: *N-hydroxyethyl* acrylamide

a) Target molecular weight
b) The degree of polymerization ($X_n$)- BY NMR.
c) The molecular weight ($M$n) of the Boc-protected polymer by ^{1}H NMR.

d) Monomer's composition ratio by ^{1}H NMR.
e) The molecular weight of the Boc-protected copolymer using SEC analysis.
f) Polydispersity index ($Đ$) by SEC analysis.

# Aqueous Media Characterization
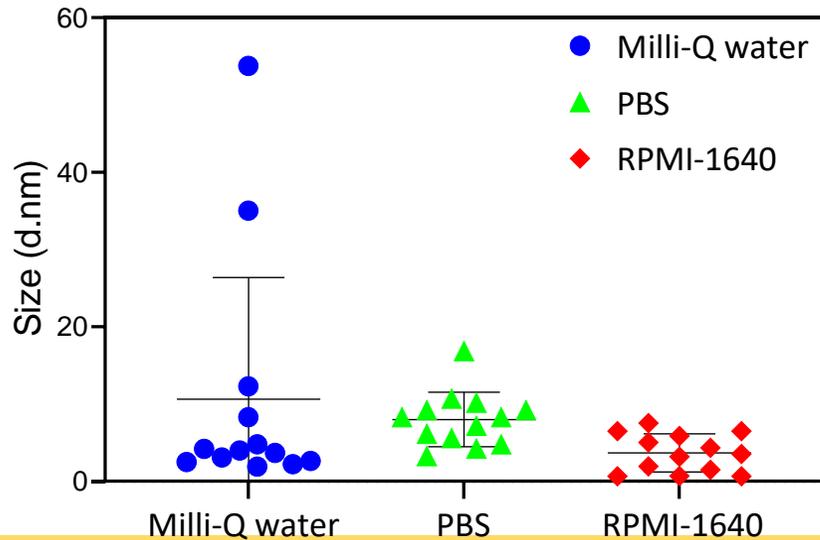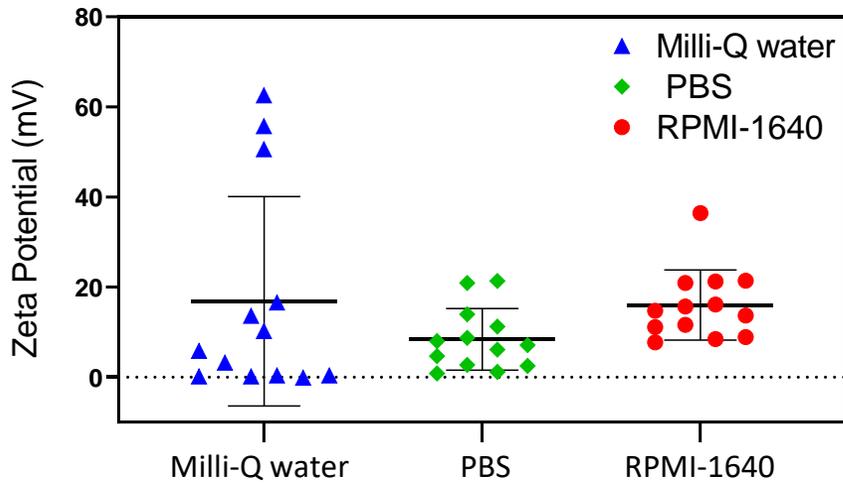


**Surface positive charge (mV)**

AB-C

C-AB

**Average size (nm)**

AB-C

C-AB
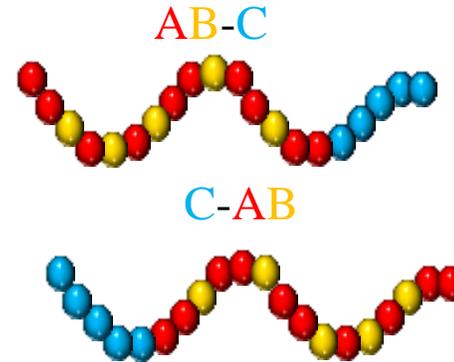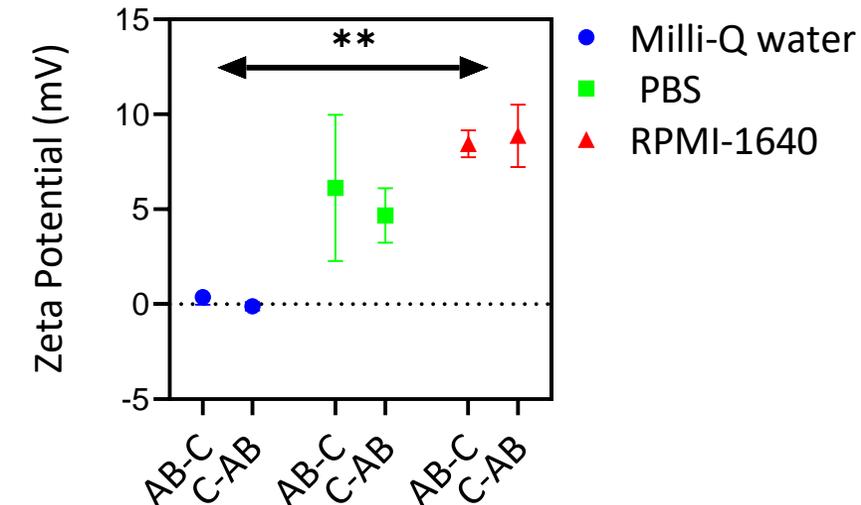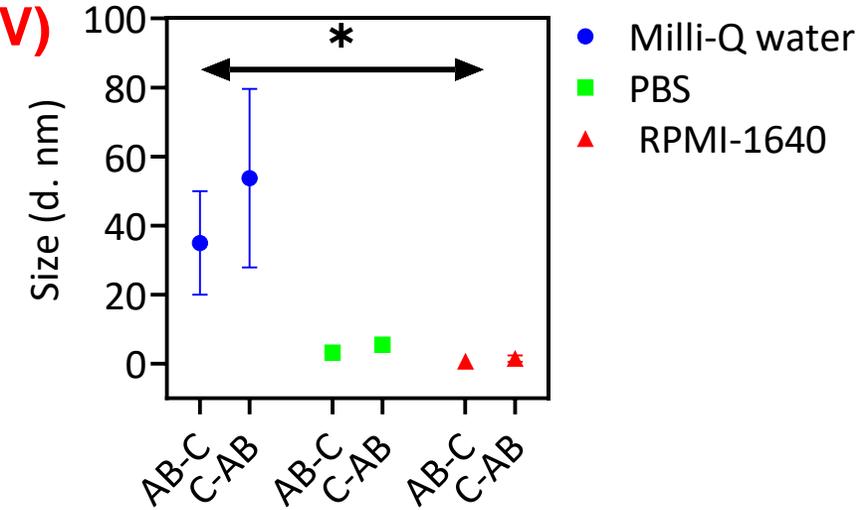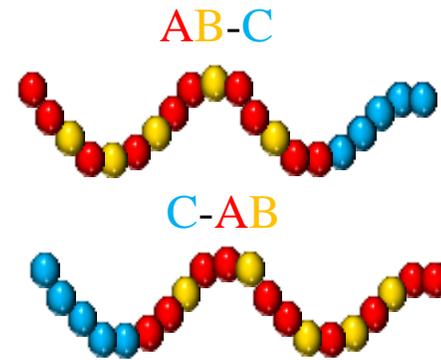
# *Targeting Candida Species*

| Structure | Sequence | *C. albicans* Lab strain | *C. albicans* Clinical isolate -1 | *C. albicans* Clincal Isolate-2 | *C. tropicalis* Clinical Isolate | *C. parapsilosis* Lab strain |
|---|---|---|---|---|---|---|
| Random | ABC | 16- 32 | 128-256 | 8-16 | 32-64 | 256 |
| Tri-block copolymers | A-B-C | 8 | 16 | 4 | 16 | 32 |
| | C-B-A | 8 -16 | 16-32 | 4 | 16 | 32 |
| | A-C-B | 64 | 128 | 16-32 | 128-256 | 64-128 |
| | B-C-A | 32-64 | 128 | 16 | >256 | 64-128 |
| | B-A-C | 64 | 128-256 | 16-32 | 128-256 | 64-128 |
| | C-A-B | 64 | 128-256 | 16 | 128 | 64-128 |
| Di-Block copolymers | AB-C | 32 | 128-256 | 8 -16 | 16-32 | 128-256 |
| | C-AB | 32-64 | 256 | 8-16 | 32 | 256 |
| | A-BC | 16 | 16 | 4 | 32 | 32 |
| | BC-A | 8 -16 | 16 | 4-8 | 32 | 32 |
| | AC-B | 16 | 16 | 4 | 16-32 | 16 |
| | B-AC | 16 | 32 | 4-8 | 32 | 32 |
| Anti-fungal drugs | Fluconazole | 0.25 | 0.25 | 0.25 | 8 | 2-4 |
| | Casponfungin | 0.25-0.5 | 0.5 | 0.5 | 0.25-0.5 | 1 |
| | Ampotetrcin B | 1 | 0.5-1 | 0.5-1 | 0.5-1 | 05- 1 |

**Minimum inhibitory concentration** ($MIC_{90}$, µg/mL): lowest concentration that inhibited the growth of *Candida* by more than 90 % when compared to untreated control.

*C. albicans and C. tropicalis* determined after 24 h while *C. parapsilosis* incubated for 48 h.

$MIC_{90}$ (µg/mL)
4 — >256

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# *Selective activity for C. albicans over RBC*

| | Schematic | Ref. | MIC$_{90, 24h}$ (µg/ml) | HC$_{50\ 2}$ h (µg/ml) | Selectivity Index |
|---|---|---|---|---|---|
| Randm |  | ABC | 8-16 | >2000 | >125 |
| Triblock Copolymer |  | A-B-C | 4 | >2000 | >500 |
| |  | C-B-A | 4 | >2000 | >500 |
| |  | A-C-B | 16-32 | >2000 | >63 |
| |  | B-C-A | 16 | >2000 | >125 |
| |  | B-A-C | 16-32 | >2000 | >63 |
| |  | C-A-B | 16 | >2000 | >125 |

| | Schematic | Ref. | MIC$_{90, 24h}$ (µg/ml) | HC$_{50\ 2}$ h (µg/ml) | Selectivity Index |
|---|---|---|---|---|---|
| Di-block copolymer |  | AB-C | 8-16 | 1000 | >63 |
| |  | C-AB | 8-16 | 1000 | >63 |
| |  | A-BC | 4-8 | >2000 | >500 |
| |  | BC-A | 4-8 | 1000 | >250 |
| |  | AC-B | 4 | >2000 | >500 |
| |  | B-AC | 4-8 | >2000 | >250 |
| Anti-fungal drugs | Fluconazole | ACTL-1 | 0.25 | Non-Haemolytic | >500 |
| | Casponfungin | ACTl-2 | 0.25 | >125 | N/A |
| | Amphotericin B | ACTL-3 | 1 | 18 | 18 |
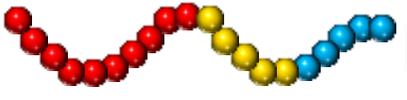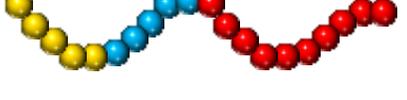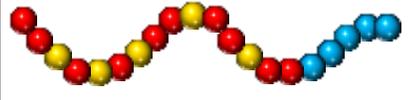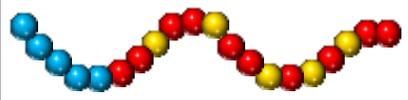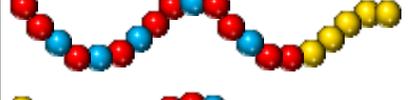
MIC$_{90}$ (µg/mL) 4 — 32  |  HC$_{50}$ 2h (µg/mL) 1000 — >2000  |  Selectivity Index 31 — >500

Selectivity index (SI: ratio of HC$_{50}$ (50% hemolytic concentration treated against RBC) against MIC of Cl-2

# *Conclusion and outlook*

✓ **Novel Synthesis;** A library of ternary well-defined multi block-sequence-controlled antifungal polymers.

✓ Altering the **arrangement** of defined block sequence results in **potent** antifungal and **increase selectivity** against tested *Candida* **species**



**PET-RAFT Polymerization**

**Promising Defined Block-sequence Antifungal Polymers**

● Cationic    ● Hydrophobic    ● Hydrophilic
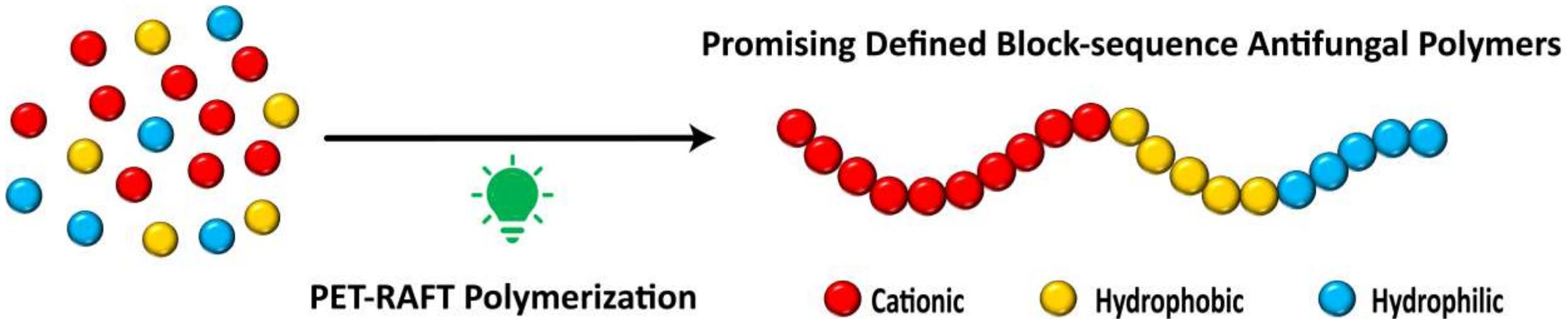
✓ Polymers against **biofilm** and their **Synergistics design approach** is under investigation.

# *Acknowledgments*

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