Coiled-coil peptide-polymer star conjugates *via* aqueous photoinduced atom transfer radical polymerization

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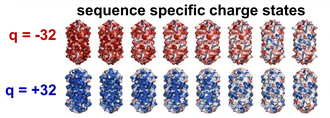
Bundlemers are a promising platform to probe peptide-polymer interactions

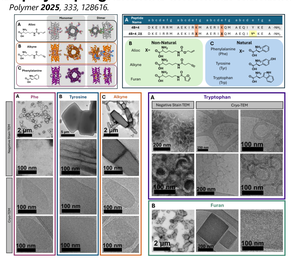
Peptide–polymer conjugates are an important class of hybrid materials that combine the molecular precision and biological functionality of peptides with the synthetic versatility of polymers. This complementary functionality has motivated the development of peptide–polymer conjugates for applications including therapeutics, drug delivery, and biosensing. **A central challenge, however, is achieving precise control not only over conjugate composition, but also over the number, placement, and molecular mass of polymer chains attached to peptide or protein-derived scaffolds. We propose bundlemers as a potential model to investigate the fundamental challenges of grafting polymers from peptide/protein surfaces.**

Evolving library of bundlemers with tunable surface functionality and environment

sequence specific charge states



spatial display of programmed chemical handles

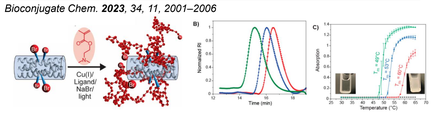


*Polymer **2025**, 333, 128676.*

*Biomacromolecules **2022** 23 (4): 1652–1661*

Original work using a negative sequence to graft DMAEMA

*Bioconjugate Chem. **2023**, 34, 11, 2001–2006*



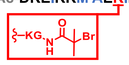
- Prior work used PhotoATRP at 365 nm to create pH-temperature-responsive DMAEMA conjugates
- Served as a vital proof-of-concept, but low peptide macroinitiator yields (average 15 mg per 0.25 mmol synth) and polymer analysis by conjugate digestion followed by traditional GPC methods using polymer standards provided limited insight

The present work reestablishes our framework to design, synthesize, and characterize bundlemer-polymer conjugates by using a higher yield bundlemer, oxygen-tolerant PICAR ATRP, an expanded monomer scope, and native SEC-UV-dRI-MALS analysis.

Bundlemer macroinitiator *via* solid-phase peptide synthesis (SPPS)

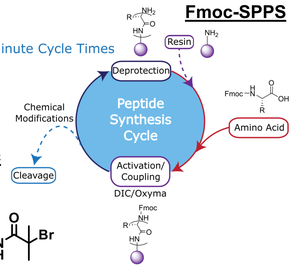
BP4-Br Peptide Sequence (75 mg/0.25 mmol synth)

Ac-DKEIRRM AEKIRKM AERIKQM AEQIYKE A-amide

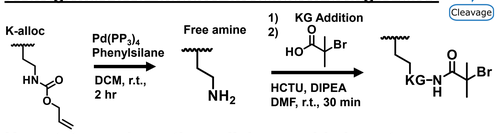


- **Hydrophobic residues** provide entropic driving force for assembly
- **Hydrophilic residues** provide stabilization through salt bridging and hydrogen bonding (parent sequence holds a net +4 charge/strand at pH 7)

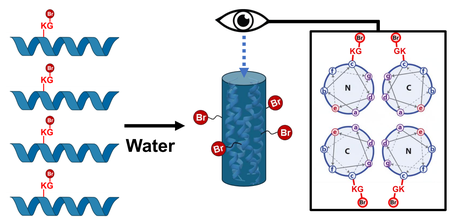
Fmoc-SPPS



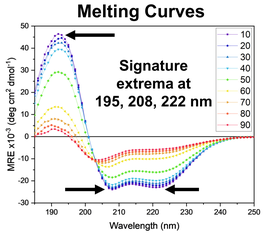
Orthogonal ATRP initiator installation using K-alloc



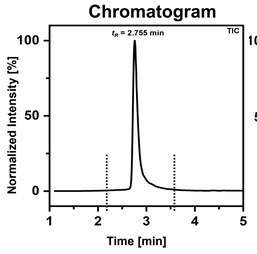
Homotetrameric, anti-parallel assembly in water



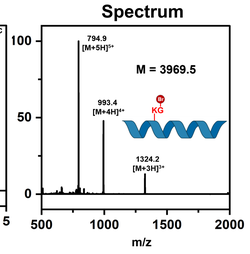
Circular Dichroism Melting Curves



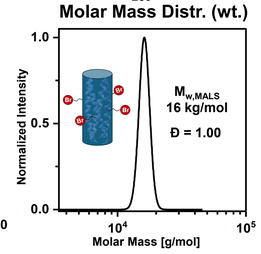
UPLC TIC Chromatogram



+ESI-TOF Mass Spectrum

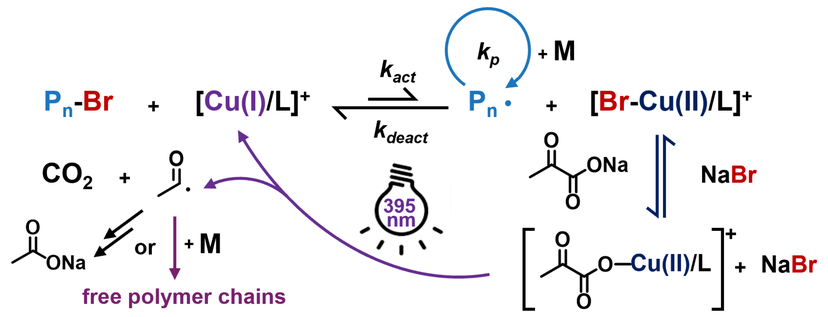


SEC-UV$_{280}$-dRI-MALS Molar Mass Distr. (wt.)

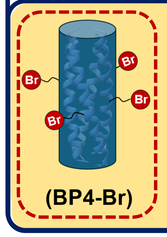


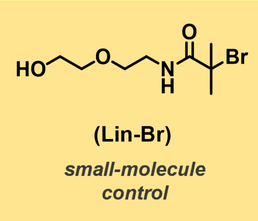
Sodium pyruvate-mediated aqueous PICAR ATRP[1]

[1]System adapted from Szczepaniak *et al., Chem. Sci.* ***2020,*** *11 (33): 8809–8816.*

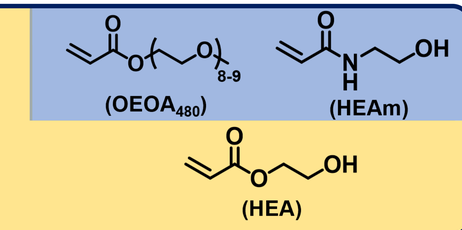


Initiator Structures



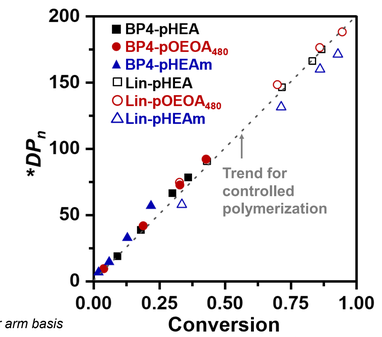


Investigated Monomers



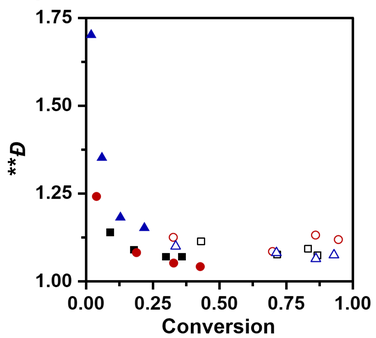
BP4-Br initiated polymerizations yield controlled star conjugates for each monomer

Polymer DP_n Evolution with Conversion



**per arm basis*

Polymer *Đ* Evolution with Conversion

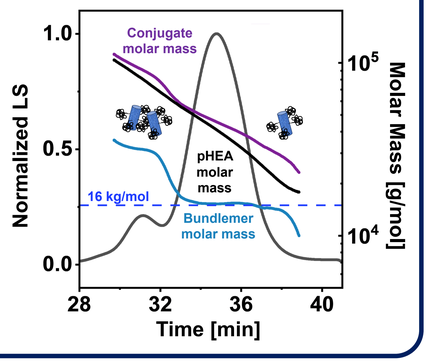


***total polymer contribution only; BP4 contribution excluded to prevent bias*

Final polymer molar masses and conversions

Polymerization	Conversion (%)	$M_{w,MALS}$ (kg/mol)**	$Đ_{polymer}$**
BP4-pHEA	36	38.8	1.07
BP4-pOEOA$_{480}$	43	184.0	1.04
BP4-pHEAm	22	30.0	1.15
Lin-pHEA	87	21.8	1.07
Lin-pOEOA$_{480}$	95	101.0	1.12
Lin-pHEAm	93	20.9	1.07

SEC-UV$_{280}$-dRI-MALS Conjugate Analysis of BP4-pHEA (60 min)

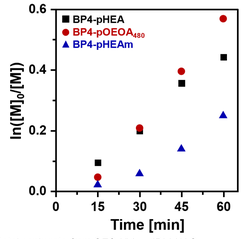


Polymerization conditions

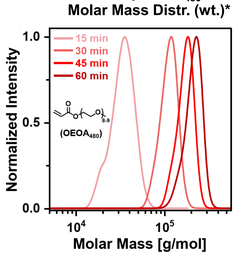
[M]/[I]/[CuBr$_2$]/[Me$_6$TREN]/[sodium pyruvate]/[NaBr] = 400/2/0.2/1.2/50/210 mM in 10 mM phosphate buffer, pH 7.4, containing 5 vol% (7% for Lin-Br) dimethylformamide (DMF). Polymerizations were performed at a nominal formulation volume of 500 µL in septum-sealed 2 mL HPLC vials, purged with nitrogen (needle in headspace, not liquid; no stirring) for 5 min, then irradiated at 395 nm, 2.5 mW cm^{-2} at 22 °C with stirring (500 rpm) for 60 min.

BP4-Br polymerization with HEAm and OEOA$_{480}$ indicate nuanced conjugate interactions

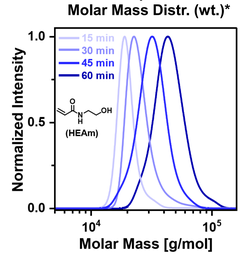
^{1}H NMR Polymerization Kinetics



BP4-pOEOA$_{480}$ Molar Mass Distr. (wt.)*



BP4-pHEAm Molar Mass Distr. (wt.)*

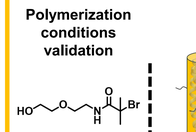


**total conjugate from SEC-UV$_{280}$-dRI-MALS*

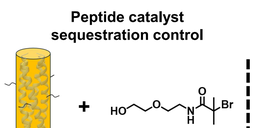
- **pOEOA$_{480}$ grafts stabilize conjugates** against aggregation, akin to PEG conjugation
- **We hypothesize** early HEAm oligomeric arms interact with the bundlemer backbone *via* favorable H-bonds, reducing ω-chain end access (subject of future work)

Control experiments with HEA suggest a local peptide surface effect decreases polymerization rate, but not control

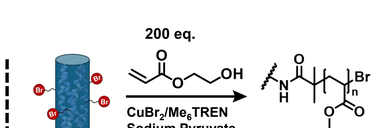
Polymerization conditions validation



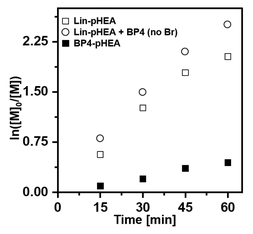
Peptide catalyst sequestration control



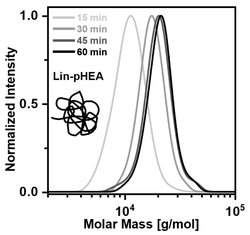
200 eq.



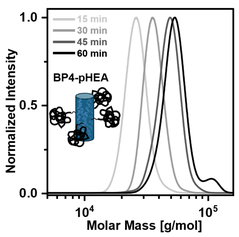
^{1}H NMR Polymerization Kinetics



Lin-pHEA Molar Mass Distr. (wt.)

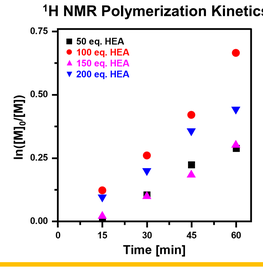


BP4-pHEA Molar Mass Distr. (wt.)

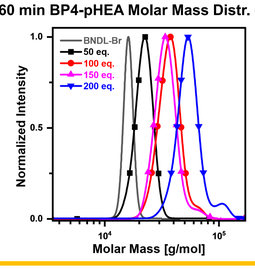


Varying HEA equivalents to yield BP4-pHEA conjugates of varying arm length

^{1}H NMR Polymerization Kinetics



60 min BP4-pHEA Molar Mass Distr. (wt.)



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