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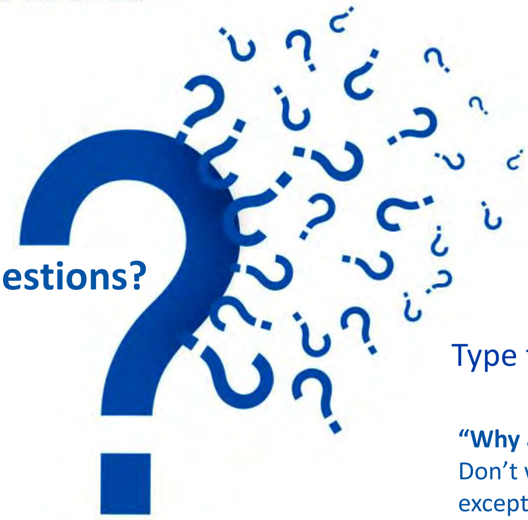

Type them into questions box!

"Why am I muted?"
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We have a collection of career resources to support you during this global pandemic:

Professional Education

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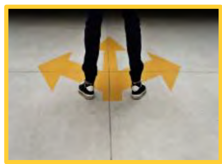

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Free Upcoming ACS Webinars!

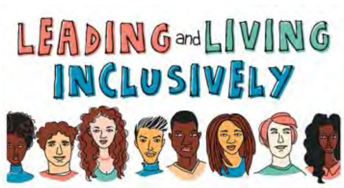
Friday, August 7, 2020 at 2-3pm ET
Speaker: Sandra Long, Post Road Consulting
Moderator: Tom Halleran, American Chemical Society

Register for Free!

What You Will Learn

- How to best position yourself on LinkedIn using effective language and visuals
- How to optimize your profile for search
- How to leverage the newest profile settings and features

Co-produced with: ACS Career Navigator



Wednesday, August 12, 2020 at 2-3pm ET
Speaker: La'Wana Harris, La'Wana Harris, Inc.
Moderator: Paula Christopher, American Chemical Society

Register for Free!

What You Will Learn

- How to equip yourself to become a more thoughtful and effective ally for advancing diversity, equity, and inclusion
- How to enable yourself to create a strategy for leveraging your power and privilege to foster greater access, opportunity, and accountability
- How to empower yourself to start leading and living inclusively by demystifying the "how" of everyday behaviors at the individual, structural, and systemic levels

Co-produced with: ACS Department of Diversity Programs and ACS Diversity, Inclusion & Respect Advisory Board

Thursday, August 13, 2020 at 2-3pm ET
Speakers: Robin Izzo, Princeton University and ACS CHAS / Frankie Wood-Black, Sophic Pursuits
Moderator: Ralph Stuart, Keene State College and ACS CCS

Register for Free!

What You Will Learn

- Advantages and challenges of using personal protective equipment for community protection
- Best practices in physical distancing and peer coaching in the teaching and research settings
- Ventilation considerations in managing COVID concerns

Co-produced with: ACS Division of Chemical Health and Safety ACS Committee on Chemical Safety

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Diana Gerbi, 2018 POLY Chair
3M(retired)

"….the next generation of polymer scientists is where we put a lot of our focus and we've really established a tremendous network of scientists at all points in their career. …our more seasoned members are active in helping support and foster the growth of the next generation through mentoring and a very active awards program."

Marc Hillmyer, 2017 POLY Chair
University of Minnesota

"…. as the university relations manager, I knew I would need to connect with a wide variety of professors and students. The Division of Polymer Chemistry provided the perfect environment to build these connections."

Karl Haider, 2016 POLY Chair
Covestro

www.polyacs.org

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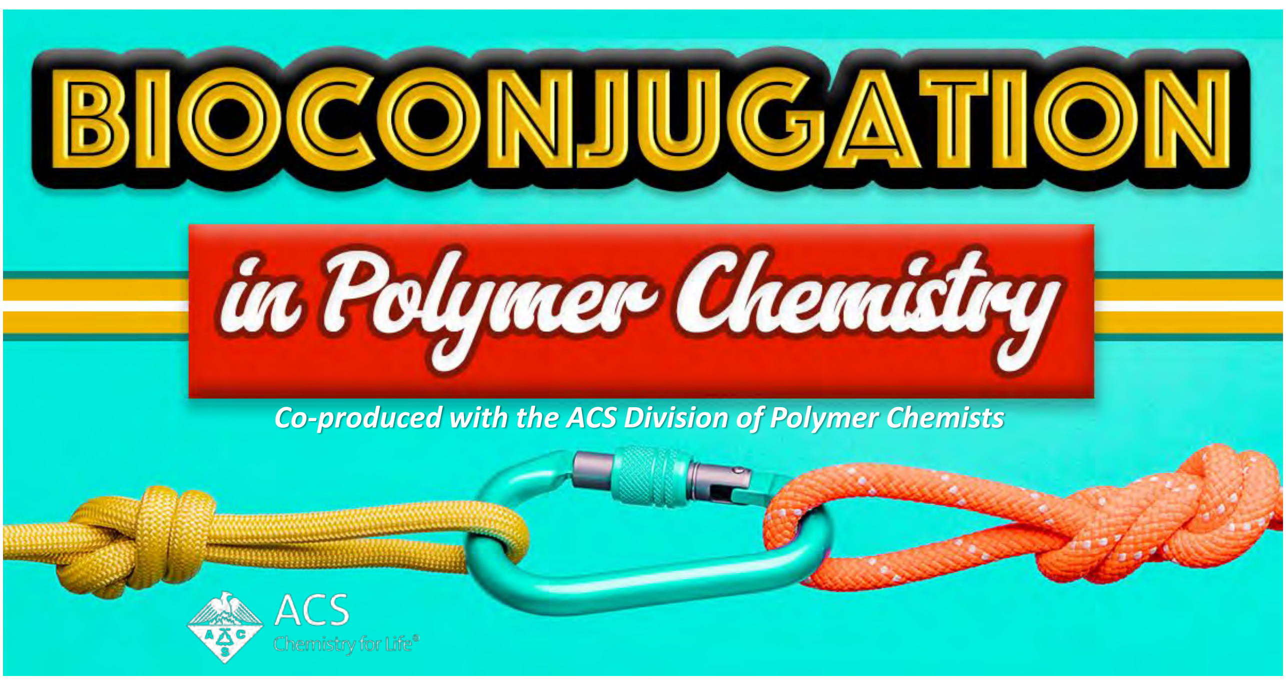

THIS ACS WEBINAR WILL BEGIN SHORTLY...

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Bioconjugation in Polymer Chemistry

Laura Stratton
Director R&D of Polymer Chemistry Innovations

Coray Colina
University Term Professor, Professor of Chemistry and Affiliate Professor of Materials Science and Engineering and Nuclear Engineering, University of Florida

Theresa Reineke
Distinguished McKnight University Professor, University of Minnesota and Associate Editor, *ACS Macro Letters*

Presentation slides are available now! Edited recordings are an exclusive ACS member benefit.

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This ACS Webinar is co-produced with ACS Division of Polymer Chemists.

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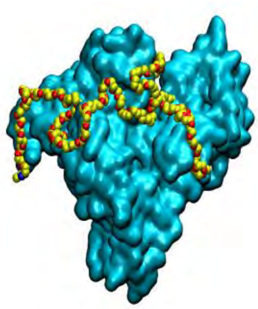
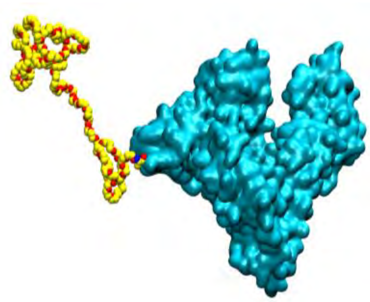
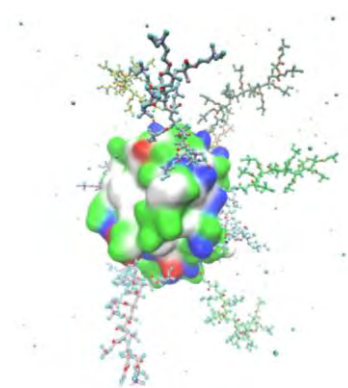
Bioconjugation in Polymer Chemistry:
an in Silico Perspective







Coray M. Colina

UF UNIVERSITY of FLORIDA

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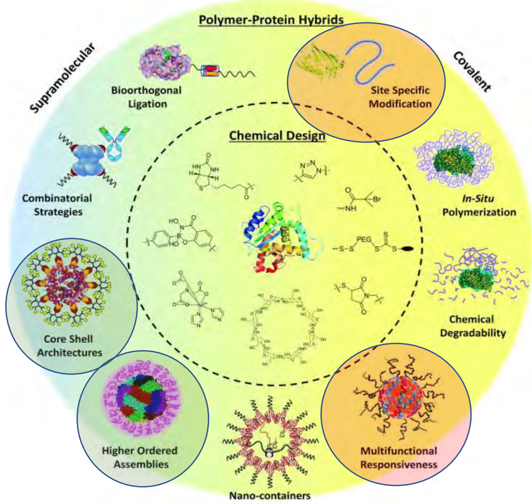
Bioconjugates

❑ Formation of a bond between a biomolecule and another molecule

noncovalent

covalent



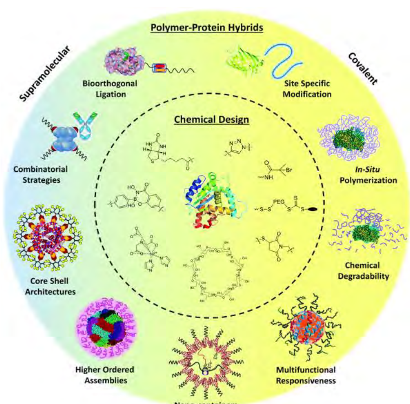
Coray Colina

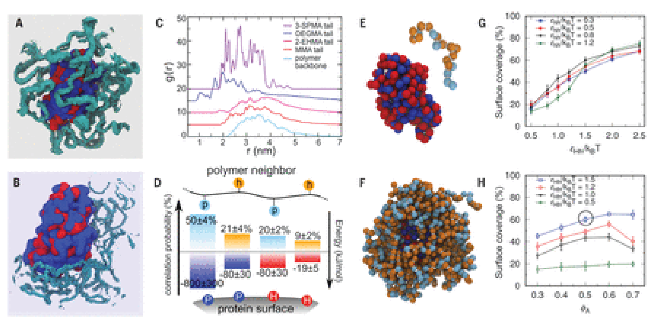
Theresa Reineke

Wu ,*Ng, Kuan, Tanja* ***Weil****, Biomater. Sci.*, 2015,3, 214-230 *2015*

Bioconjugates

❑ Formation of a bond between a biomolecule and another molecule





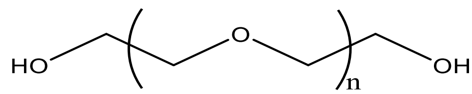
Wu ,*Ng, Kuan, Tanja* ***Weil****,*
Biomater. Sci., 2015,3, 214-230 *2015*

Panganiban, Qiao,…, Monica **Olvera de la Cruz**
Ting **Xu** *Science,* 2018, 359, 6381, 1239-1243

Benefits of protein polymer conjugates

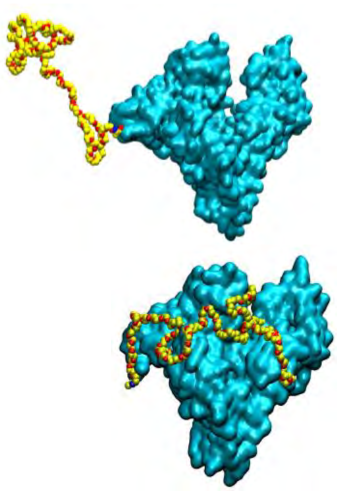
- Biocompatible
- Fine tune solubility
- Ability to readily diffuse
- Targeted drug delivery
- Stimuli responsive

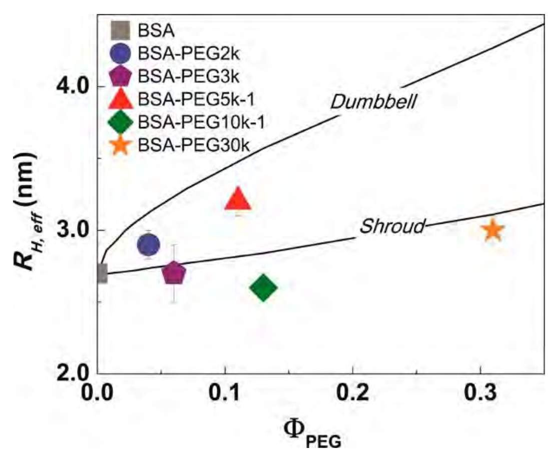
Polyethylene glycol (PEG)



PEGylation

PEG – protein interactions



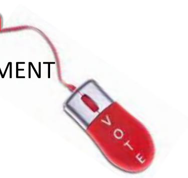


Munasinghe, A.; et al. *J. Phys. Chem.B* **2019,** *123* (25) , 5196-5205.

Ferebee, R.; et al. *J. Phys. Chem. B* **2016**, *120* (20), 4591–4599

Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

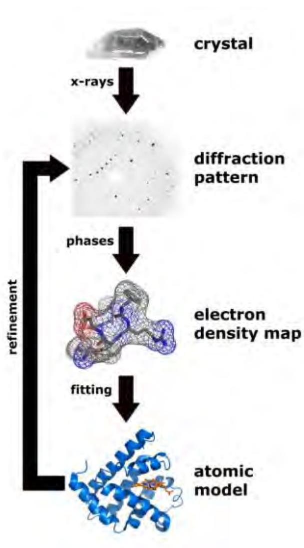


Computational models able to provide explanations on how polymers interact with a protein require? (Select all that apply)

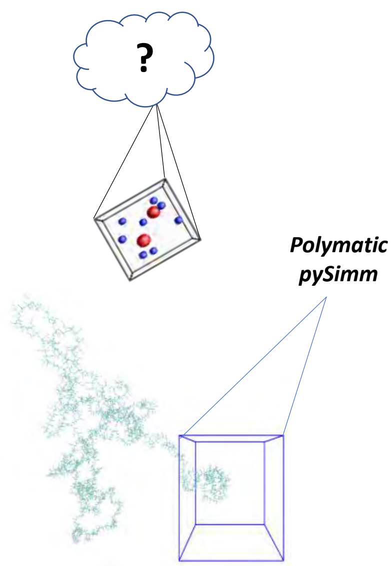
- Experimental protein structure (e.g. X-ray, NMR)
- Experimental polymer structure
- Force field/interatomic potential selection
- Computational software/codes
- Simulations, analysis and validation

** If your answer differs greatly from the choices above tell us in the chat!*

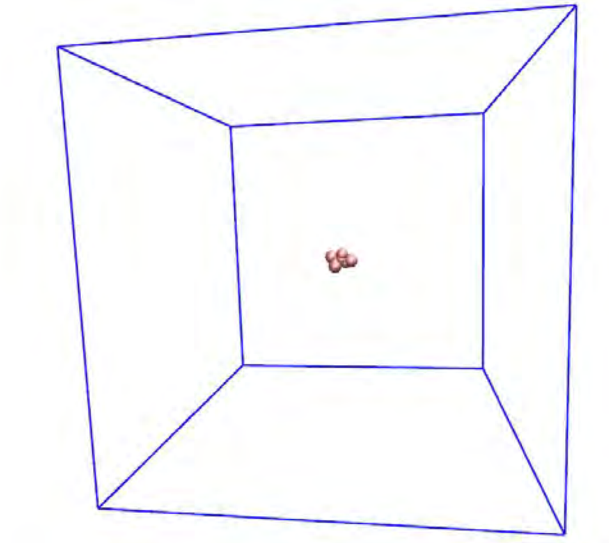
Structure generation (in silico synthesis)
At the atomistic level

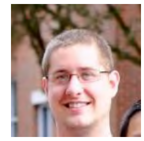


Thomas Splettstoesser
(www.scistyle.com)



Structure generation (in silico synthesis)
At the atomistic level





Mike Fortunato

Laurent Abbott
polymatic

http://pysimm.org/

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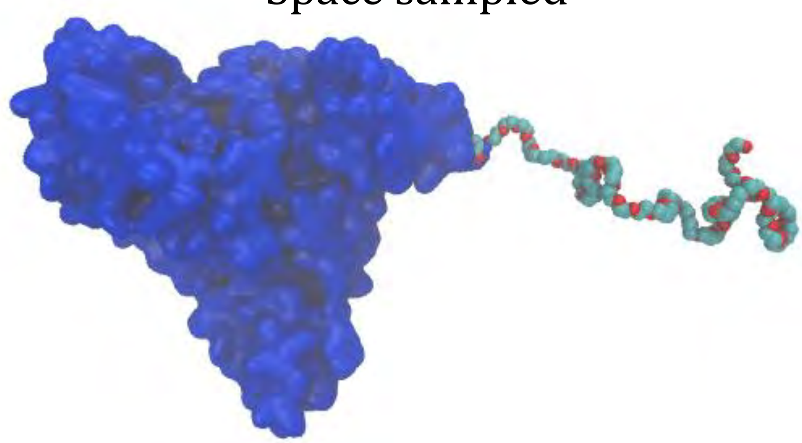
PEGylation at the N-terminal
Space sampled



Aravinda Munasinghe

Akash Mathavan

Akshay Mathavan

2k PEG

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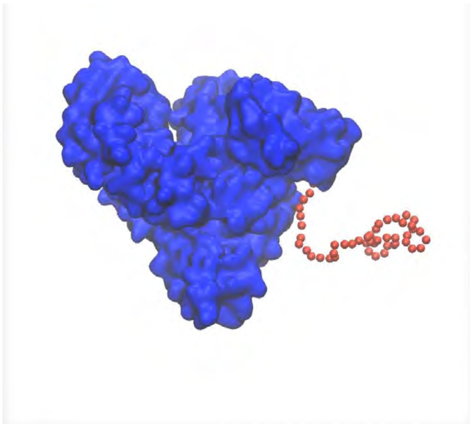
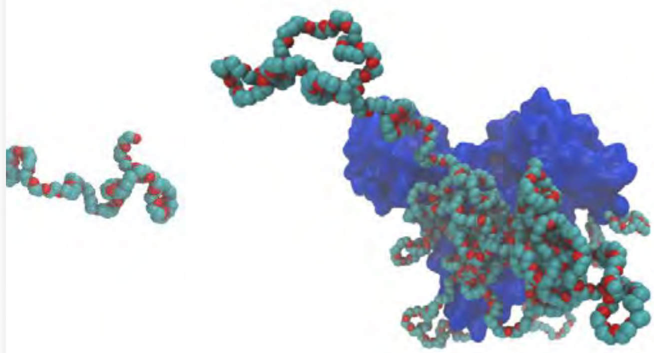
PEGylation at the N-terminal
Space sampled

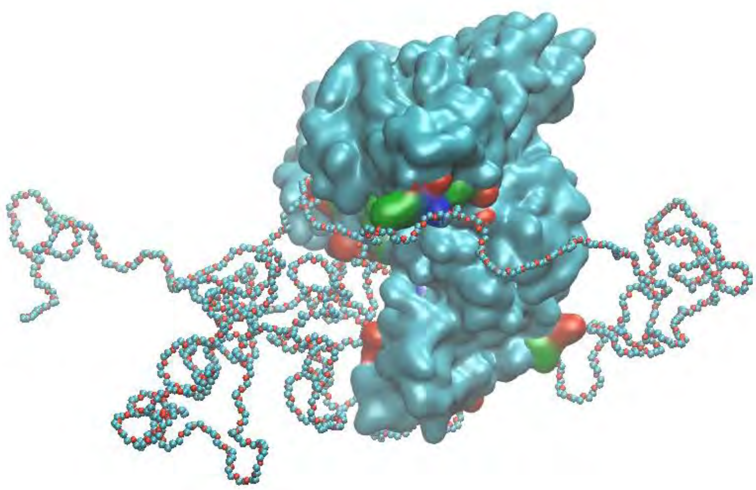




2k PEG

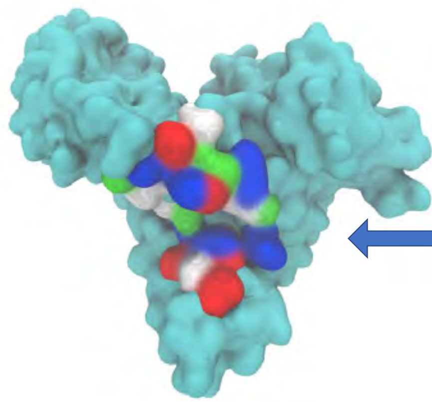
20k PEG

20k-PEG at the N-terminal



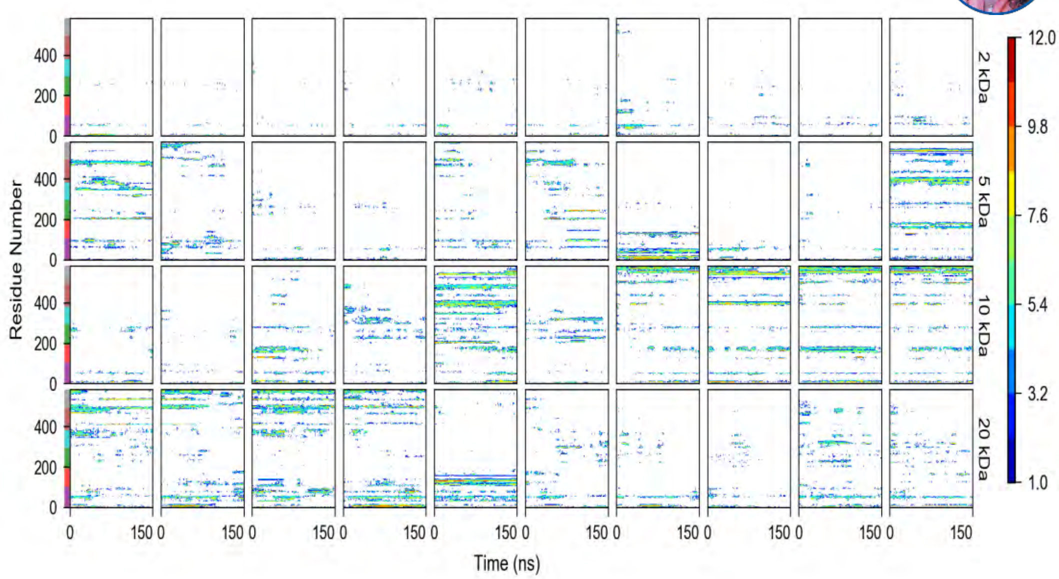
Hydrophobic Hydrophilic Positively charged Negatively charged

PEG-Protein Fragment interactions in BSA-20k N-terminal



Res name	Res ID	Normalized time
GLU	478	0.99
CYS	486	0.99
CYS	475	0.95
LYS	474	0.95
SER	479	0.95
ALA	489	0.92
ASN	482	0.91
LYS	471	0.90
THR	477	0.90
PRO	485	0.88
LEU	490	0.88
LYS	350	0.87
LYS	204	0.87
PHE	205	0.87
GLU	470	0.86
GLU	351	0.82
THR	491	0.80
PHE	487	0.79
THR	473	0.77
ARG	347	0.75

PEGylation at the N-terminal

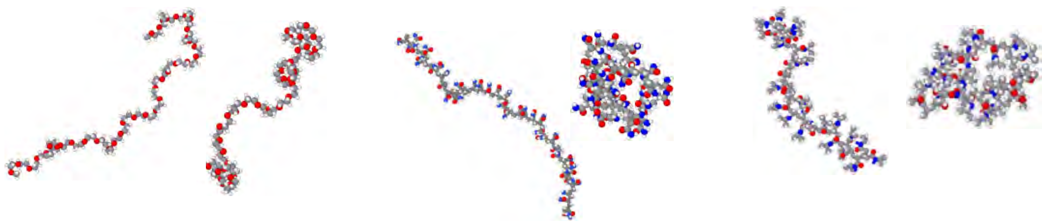


Munasinghe, et al. JPCB, 2019, 123, (25) 5189-5384

Beyond PEGylation

Simulation snapshots illustrating polymer conformations:
Single polymer chain in water

PEO/PEG   PAM   PNIPAAm



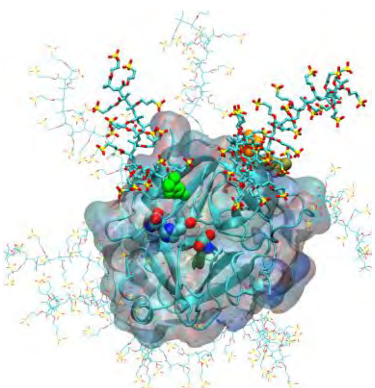
Shalini Jayaraman Rukmani

Grit Kupgan

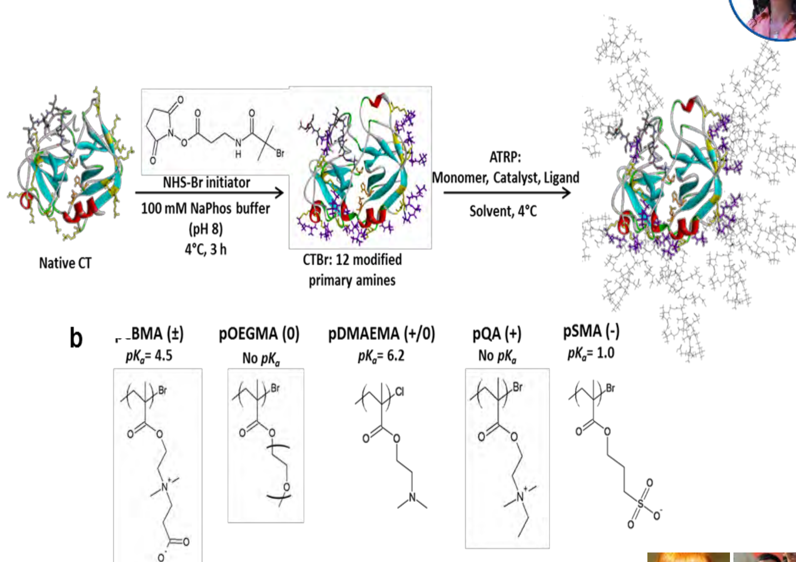
Dylan Anstine

Rukmani, S. J., Kupgan, G., Anstine, D. M., Colina, C. M., "A molecular dynamics study of water-soluble polymers: analysis of force fields from atomistic simulations" *Molecular Simulation*, 45, 310-321, **2018**

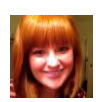
Beyond PEGylation

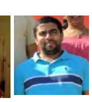


CT-pSMA

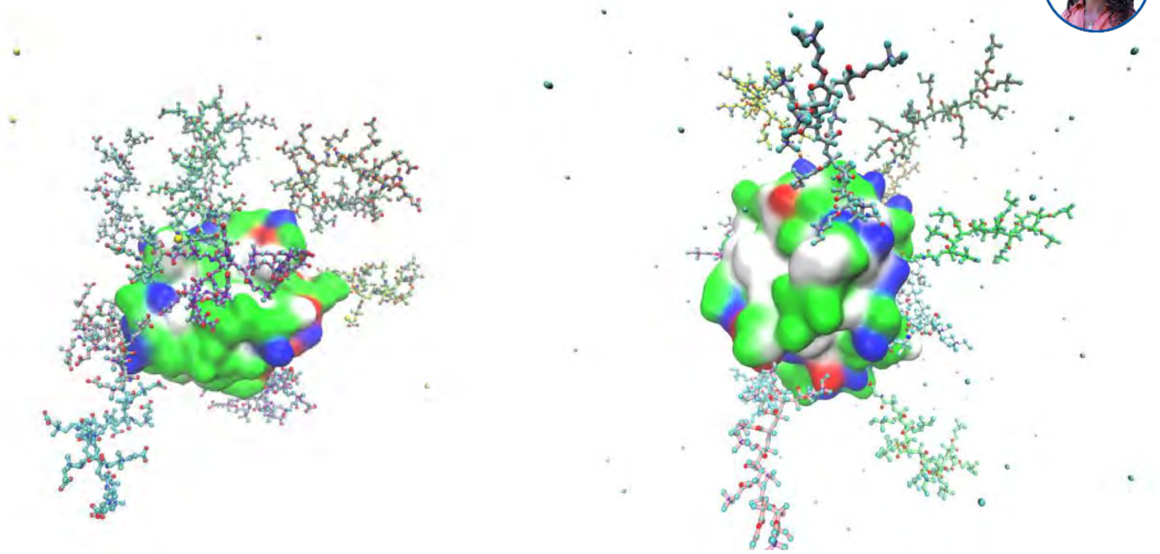


Baker, S., Munasinghe, A., Murata, H., Lin, P., Matyjaszewski, K., Colina, C. M., Russel, A. J., Biomacromolecules, **2018**, 19(9), 3798-3813



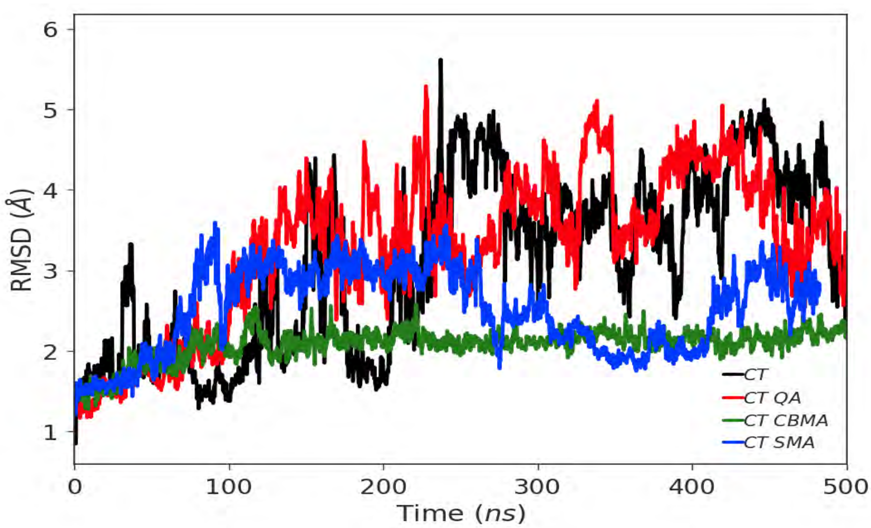


Chymotrypsin-polymer bioconjugates

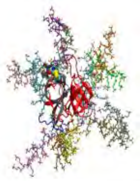


Baker, S., Munasinghe, A., Murata, H., Lin, P., Matyjaszewski, K., Colina, C. M., Russel, A. J., Biomacromolecules, **2018**, 19(9), 3798-3813

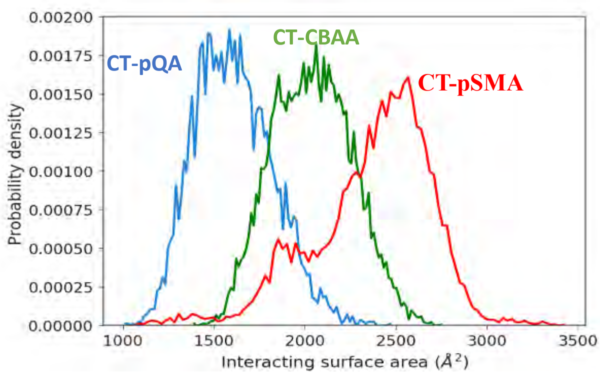
Chymotrypsin-polymer bioconjugates

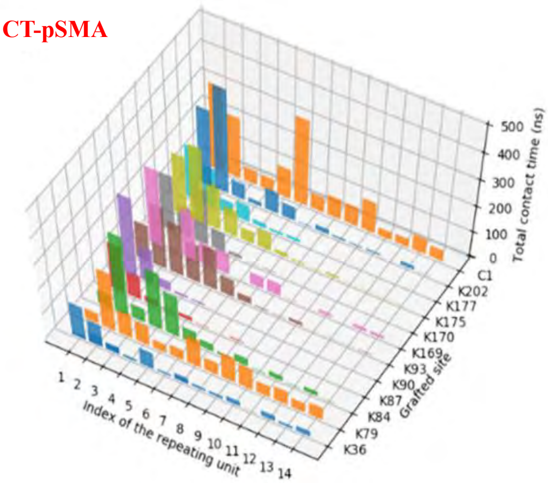


Baker, S., Munasinghe, A., Murata, H., Lin, P., Matyjaszewski, K., Colina, C. M., Russel, A. J., Biomacromolecules, **2018**, 19(9), 3798-3813



Chymotrypsin-polymer bioconjugates





Munasinghe, A., Baker, S. L., Lin, P., Russell, A. J., Colina, C. M., "Structure-Function-Dynamics of α-Chymotrypsin based Conjugates as a Function of Polymer Charge" *Soft Matter*, **2020**, 16, 456-465.

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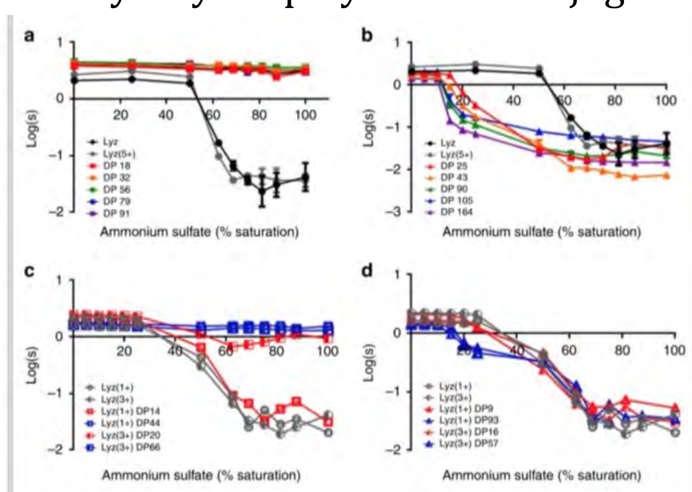
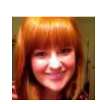
Tuning the salt solubility of proteins

Lysozyme-polymer bioconjugates



Bibifatima Kaupbayeva

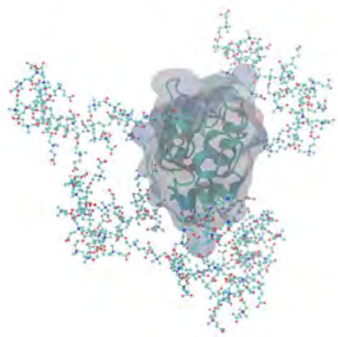


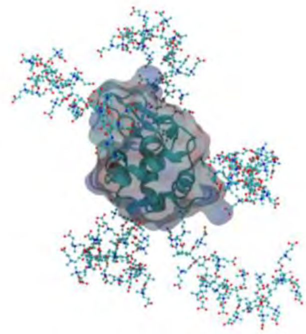
Stefanie Baker

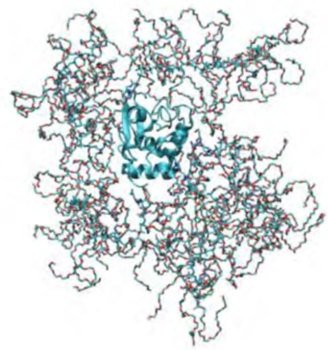
UF UNIVERSITY of FLORIDA

Baker, S. L., Munasinghe, A., Kaupbayeva, B., Kang, N. R., Certiat, M., Murata, H., Matyjaszewski, K., Lin, P., Colina, C. M., Russell, A. J., *Nat.* Commun., **2019**, 10, 4718.

Tuning the salt solubility of proteins







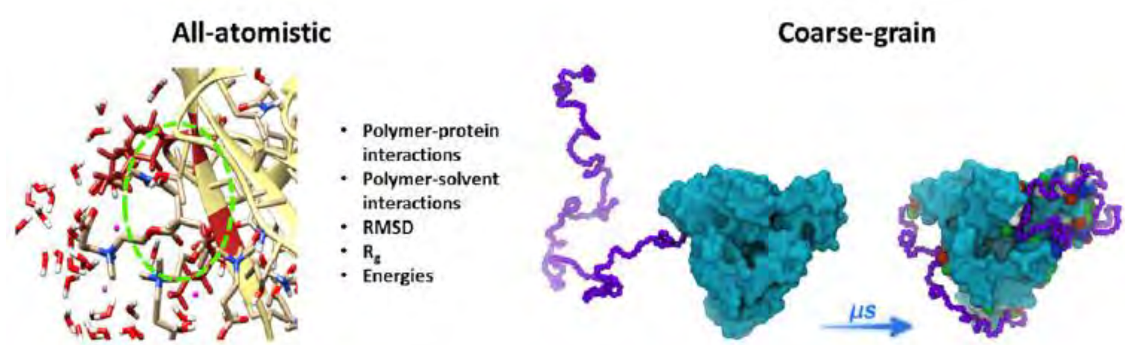
pCBMA in 0.0M NaCl

pCBMA in 5.0M NaCl

pOEGMA in 0.0M NaCl
pOEGMA in 5.0M NaCl

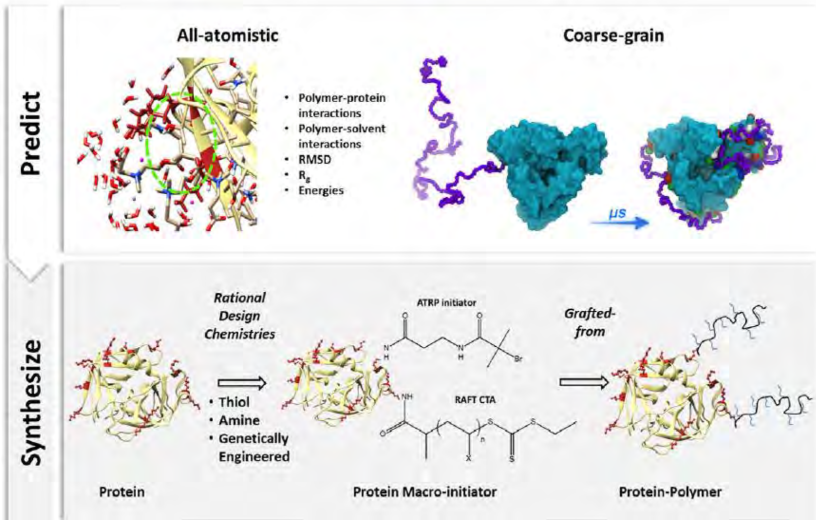
Baker, S. L., Munasinghe, A., Kaupbayeva, B., Kang, N. R., Certiat, M., Murata, H., Matyjaszewski, K., Lin, P., Colina, C. M., Russell, A. J., *Nat.* Commun., **2019**, 10, 4718.

Bioconjugates *in silico*
Challenges and Outlook



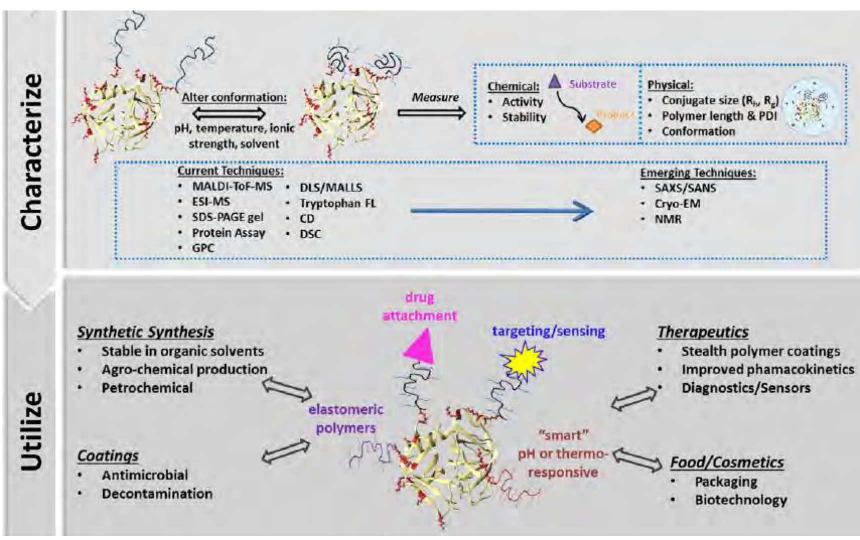
Lin, P. and C. M. Colina, Molecular Simulation of Protein-Polymer Conjugates, Curr. Opin. in Chem. Eng. **2019,** 23, 1-7

Experimental and Computation



Russel, A. J., Baker, S., Colina, C. M., Figg, C. A., Kaar, J. L., Matyjaszewski, K. Simakova, A., Summerlin, B. S., "Next generation protein-polymer conjugates" AIChE Journal, **2018**, 64(9), 3230-3245

Experimental and Computation



Russel, A. J., Baker, S., Colina, C. M., Figg, C. A., Kaar, J. L., Matyjaszewski, K. Simakova, A., Summerlin, B. S., "Next generation protein-polymer conjugates" AIChE Journal, **2018**, 64(9), 3230-3245

Acknowledgments

Winter 2017

Winter 2020

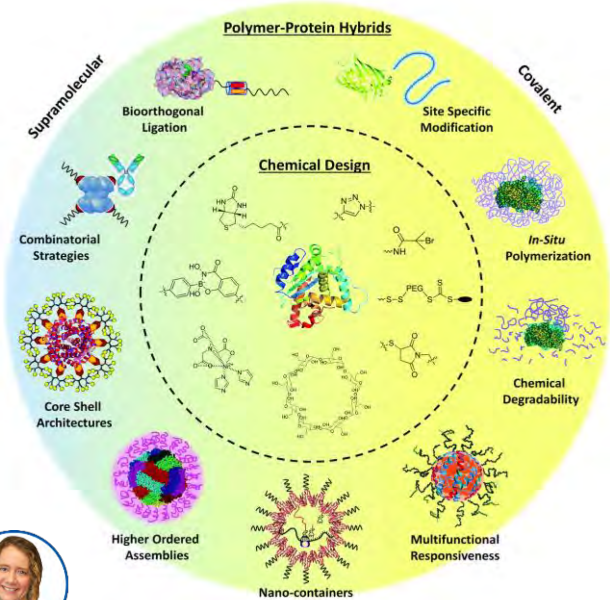
NSF
ENERGY.GOV
NIH National Institutes of Health
Turning Discovery Into Health
UF UNIVERSITY of FLORIDA
Preeminence Initiative

REINEKE GROUP

Polymer Architecture Affects Bioconjugation and Delivery Performance with Therapeutic Payloads

Theresa M. Reineke
Department of Chemistry
University of Minnesota

Bioconjugation



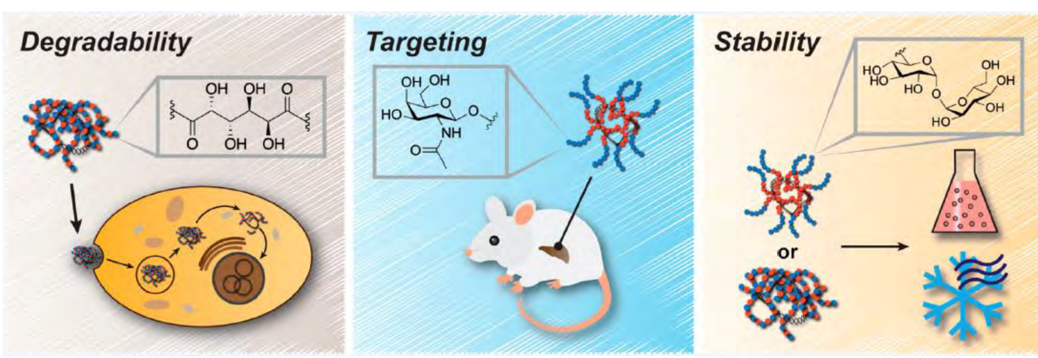
Bioconjugation is the formation of a bond between a biomolecule and another molecule

The focus of this talk is on supramolecular or noncovalent assembly of polymers with nucleic acids and proteins into:

- core-shell architectures
- higher ordered assemblies

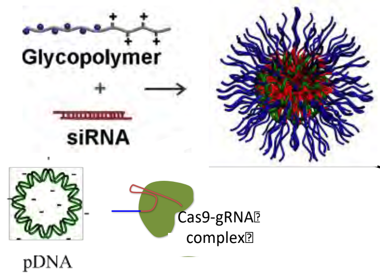
Wu, Ng, Kuan, Weil, *Biomater. Sci.* **2015**

Nucleic acid and protein bioconjugation with polymers

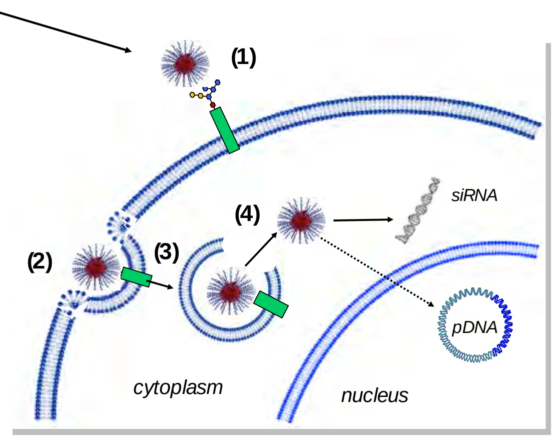


C. Van Bruggen, J. K. Hexum, Z. Tan, R. J. Dalal, T. M. Reineke *Acc. Chem. Res.* **2019**, 52, 1347–1358

Can we help biologics be affordable, stable, and readily available?

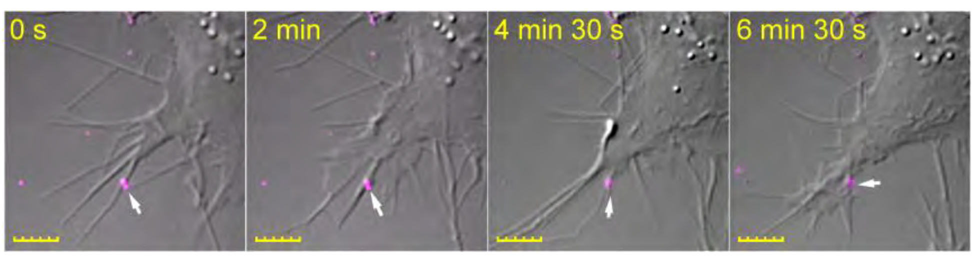


1. Cell surface binding
2. Endocytosis
3. Intracellular transport
4. Nucleic acid release

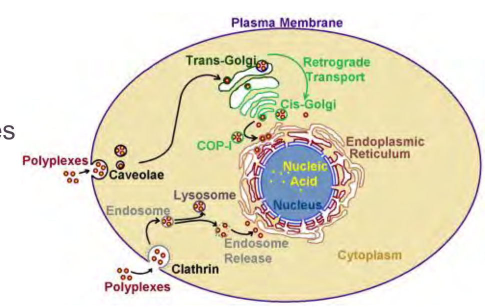


C. Van Bruggen, J. K. Hexum, Z. Tan, R. J. Dalal, T. M. Reineke, *Acc. Chem. Res.* ***2019***, *52, 1347–1358*

Polyplexes traffic in cells similar to viruses



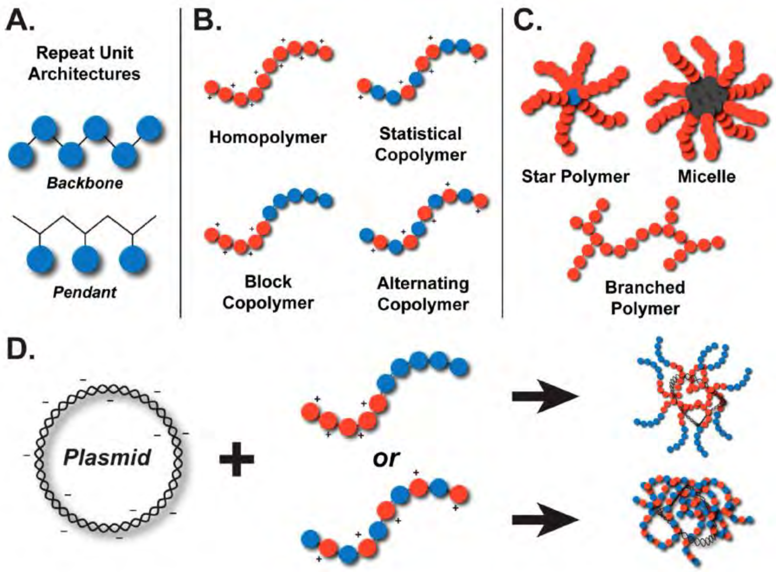
- Bind to cell surface GAGs (heparan sulfate)
- Internalized by filipodia into caveolae and clathrin vesicles
- Trafficked to the Golgi along actin/microtubules
- Trafficked from Golgi to ER via COP-I vesicles
- Localize heavily with ER: contiguous with nucleus



K. Fichter, N. Ingle, P. McLendon, T. M. Reineke, *ACS Nano*, **2013**, 7, 347–364

N. Ingle, J. Hexum, T. M. Reineke, *Biomacromolecules*, **2020**, 21, 1379-1392

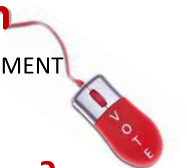
Polymer architecture plays an important role



C. Van Bruggen, J. K. Hexum, Z. Tan, R. J. Dalal, T. M. Reineke *Acc. Chem. Res.* **2019**, 52, 1347–1358

Audience Survey Question

ANSWER THE QUESTION ON BLUE SCREEN IN ONE MOMENT

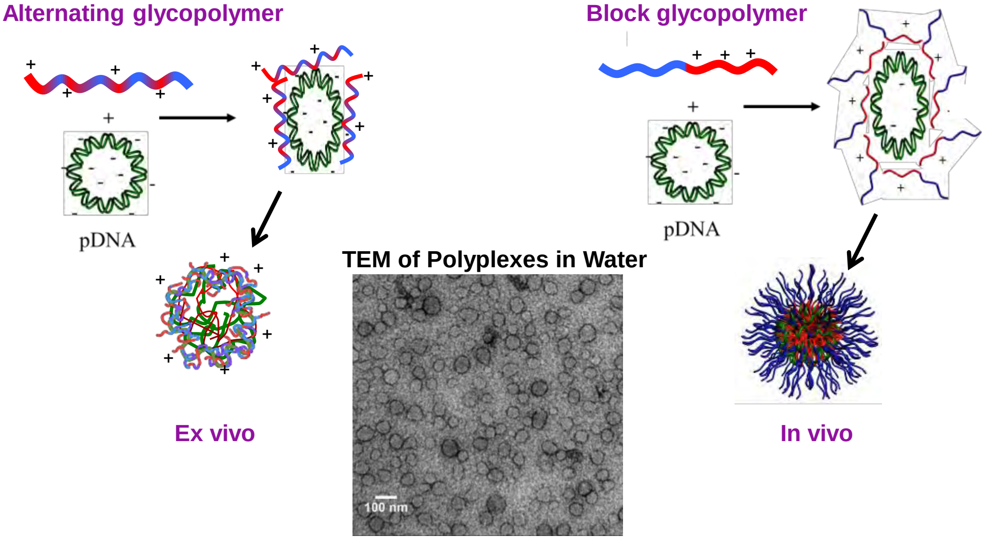


What is your favorite method to synthesize polymers?

- Step growth polymerization
- Ring opening polymerization
- Free radical polymerization
- Controlled radical: RAFT (reversible addition fragmentation chain transfer polymerization)
- Controlled radical: ATRP (atom transfer radical polymerization)

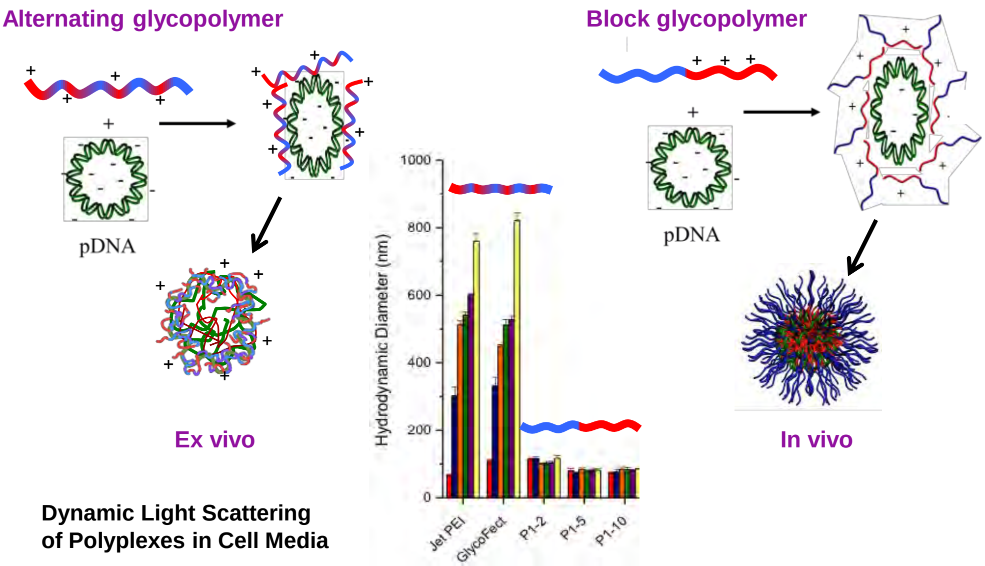
** If your answer differs greatly from the choices above tell us in the chat!*

Polymer architecture affects application



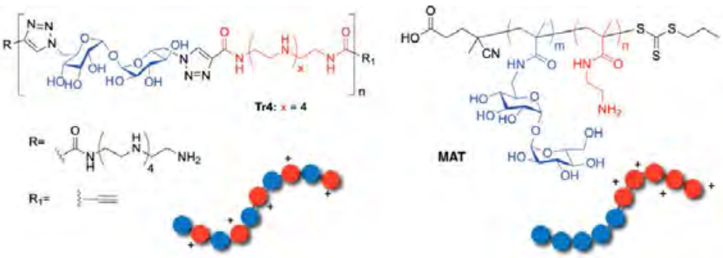
TEM of Polyplexes in Water

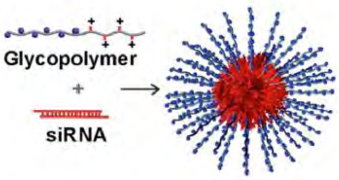
Polymer architecture affects application



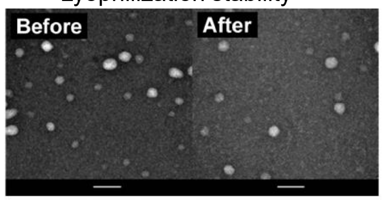
Dynamic Light Scattering
of Polyplexes in Cell Media

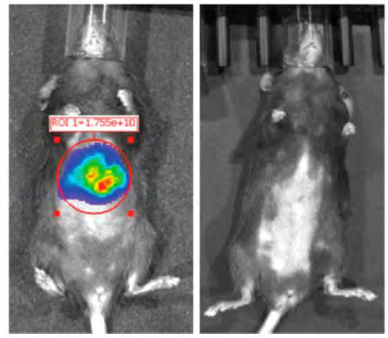
Backbone versus pendant functionality





Lyophilization stability

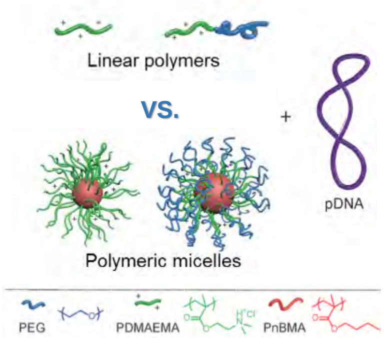




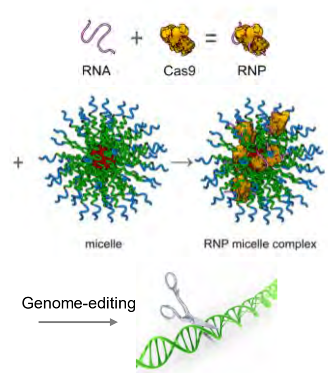
Y. Liu, T. M. Reineke, J. *Am. Chem. Soc.* **2005**, 127, 3004-3015
S. Srinivasachari, Y. Liu, G. Zhang, L. Prevette, T. M. Reineke , *J. Am. Chem. Soc.* **2006**, 128, 8176-8184
A. Sizovs, L. Xue, Z. Tolstyka, N. Ingle, Y. Wu, M. Cortez, T. M. Reineke, *J. Am. Chem. Soc.* **2013,** 135, 15417–15424
Z. Tolstyka, H. Phillips, M. Cortez, Y. Wu, N. Ingle, J. Bell, P. Hackett, T. M. Reineke, *ACS Biomaterials Sci. Eng.* **2016,** 2, 43-55

Polymer design and bioconjugate assembly

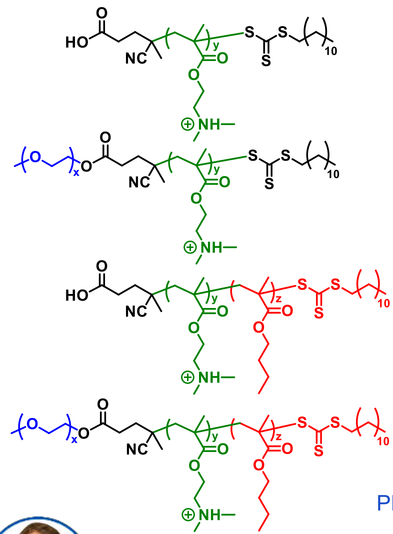
Linear polymers vs micelles for DNA conjugation and delivery: Influence of polymer architecture



Micelles for CRISPR-Cas9 ribonucleoprotein conjugation and delivery: Effective genome-editing



Comparing polyplexes to micelleplexes

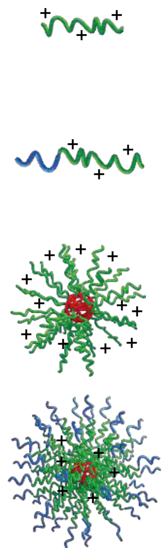


PDMAEMA (D)

PEO-*b*-PDMAEMA (OD)

PDMAEMA-*b*-PnBMA (DB)

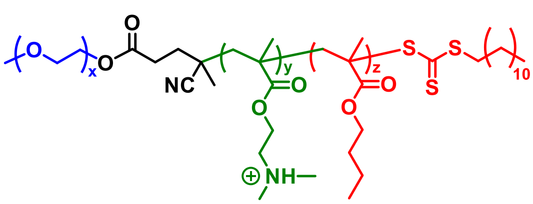
PEO-*b*-PDMAEMA-*b*-PnBMA (ODB)



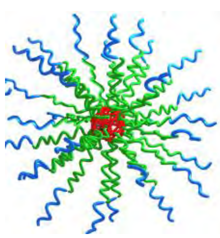
Linear polymers
↓ DNA
"Polyplexes"

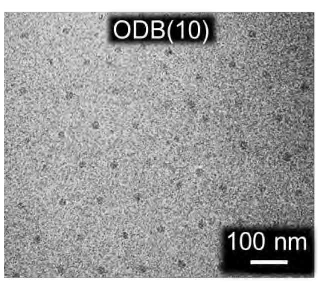
Self-assembled micelles
↓ DNA
"Micelleplexes"

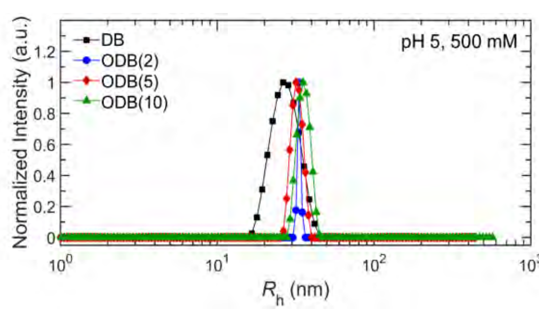
DB and ODB micelles are uniform in solution



Short name	$M_{n,O}$ (kDa)	$M_{n,D}$ (kDa)	$M_{n,B}$ (kDa)	M_n (kDa)	$Đ$
DB		27	14	41	1.10
ODB(2)	2	27	24	54	1.12
ODB(5)	5	27	25	58	1.18
ODB(10)	10	28	24	64	1.14

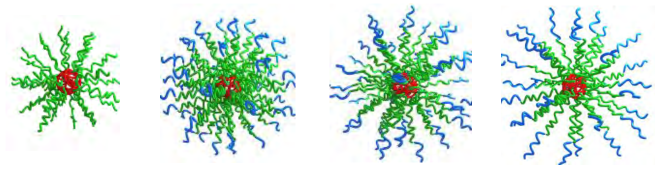






Y. Jiang, T. L. Lodge, T. M. Reineke, *J. Am. Chem. Soc.*, **2018,** 140, 11101–11111

Micelles allow quantitative characterization

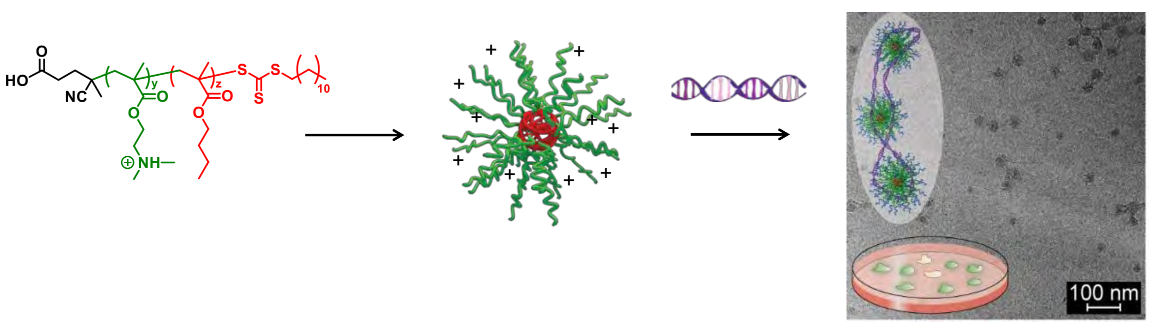


	DB	ODB(2)	ODB(5)	ODB(10)
R_h (nm) by DLS*	27	32	31	34
μ_2/Γ^2	0.02	0.002	0.01	0.02
R_c (nm) by SLS & SAXS	12 ± 1	13 ± 1	11 ± 1	11 ± 1
M_w (MDa) by SLS	8.7 ± 1.0	12.3 ± 0.2	8.9 ± 0.2	9.0 ± 0.2
N_{agg}	90 ± 10	230 ± 30	150 ± 20	140 ± 20
Amine/micelle (×10^3)	16 ± 2	40 ± 1	26 ± 1	25 ± 1

Y. Jiang, T. L. Lodge, T. M. Reineke, *J. Am. Chem. Soc.*, **2018,** 140, 11101–11111

Self-assembled micelles: a versatile architecture

Complex structure is built on the micelle architecture
Easy to control and potential co-delivery of small molecule drugs

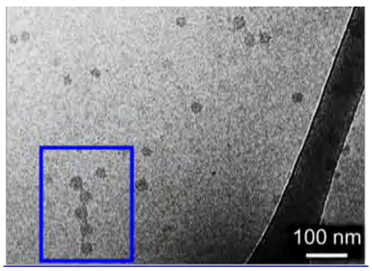


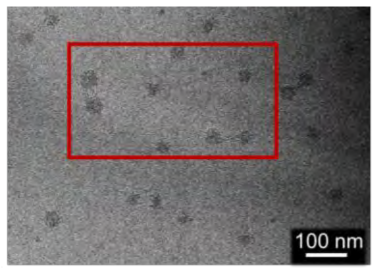
Y. Jiang, T. L. Lodge, T. M. Reineke, *J. Am. Chem. Soc.*, **2018,** 140, 11101–11111
Y. Jiang, T. M. Reineke, T. L. Lodge, *Macromolecules*, **2018** *51,* 1150-1160

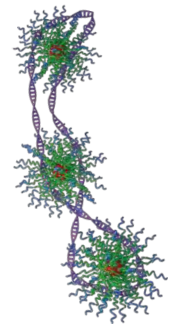
Bioconjugation can be quantitatively observed

Micelleplexes	DB	ODB(2)	ODB(5)	ODB(10)
$\#_{DNA}/\#_{micelle}$ at N/P = 5	0.62	1.6	1.0	1.0
$\#_{micelle}$/complex	4.0	3.4	2.7	2.1
$\#_{DNA}$/complex	2.5	5.3	2.7	2.1

DLS
CryoTEM

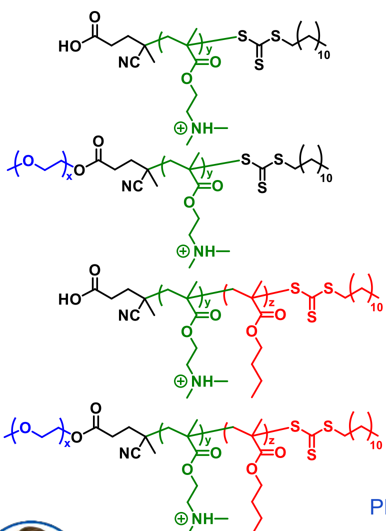






As PEG length increases, micelleplex size decreases

Comparing polyplexes to micelleplexes: bioconjugation

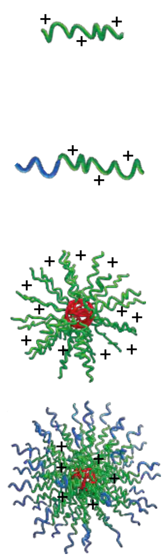


PDMAEMA (D)

PEO-*b*-PDMAEMA (OD)

PDMAEMA-*b*-PnBMA (DB)

PEO-*b*-PDMAEMA-*b*-PnBMA (ODB)



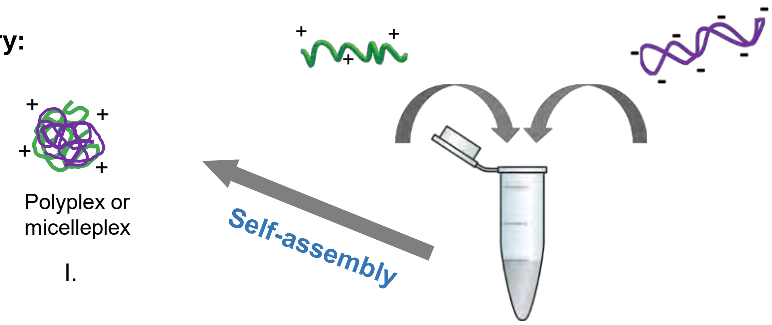
Linear polymers
↓ DNA
"Polyplexes"

Self-assembled micelles
↓ DNA
"Micelleplexes"

Bioconjugation and cellular delivery: key factors

Key factors for successful delivery:

I. Complex formation and colloidal stability



Bioconjugation and cellular delivery: key factors

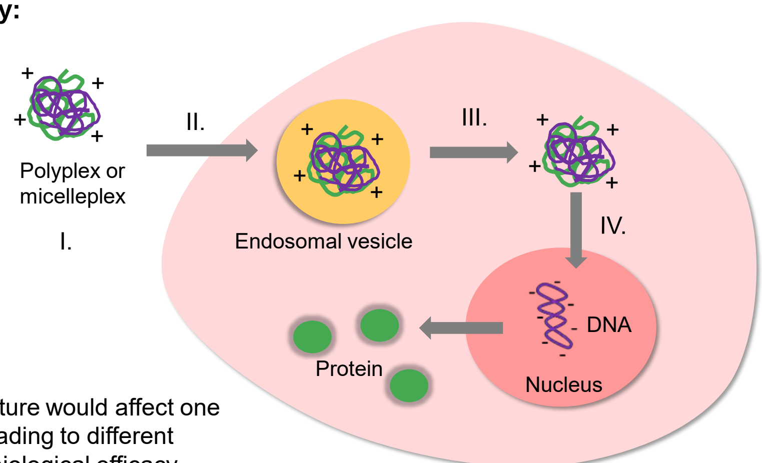
Key factors for successful delivery:

I. Complex formation and colloidal stability

II. Cellular entry efficiency and mechanisms

III. Endosomal vesicle escape

IV. DNA expression



Hypothesis: Polycation architecture would affect one or more of the above aspects, leading to different particle physical properties and biological efficacy.

Conjugation and colloidal stability

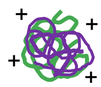
Key factors for successful delivery:

I. Complex formation and colloidal stability

II. Cellular entry efficiency and mechanisms

III. Endosomal vesicle escape

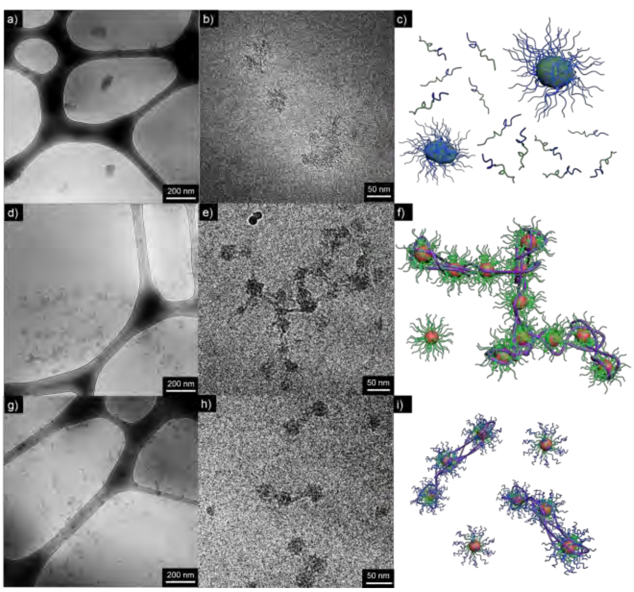
IV. DNA expression



Polyplex or micelleplex

I.

DNA conjugation and complex morphology



OD

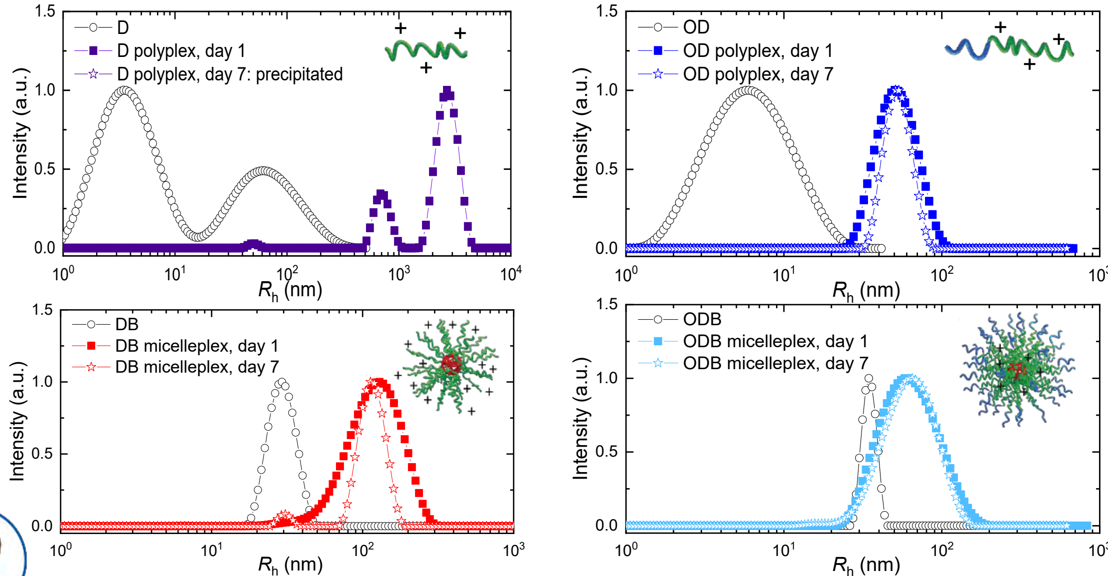
DB

ODB

- DNA complexed and condensed in the core of the core-shell structure
- DNA wraps around micelles into higher-ordered structures similar to how histones compact DNA in chromatin

Z. Tan, Y. Jiang, W. Zhang, L. Karls, T. L. Lodge, T. M. Reineke, *J. Am. Chem. Soc.* **2019** 141, 15804–15817

Polyplex/micelleplex colloidal stability over time



Cellular entry

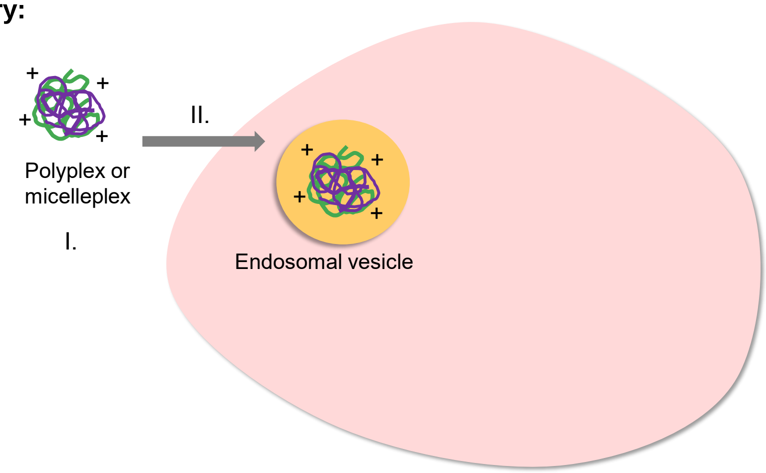
Key factors for successful delivery:

I. Complex formation and colloidal stability

II. Cellular entry efficiency and mechanisms

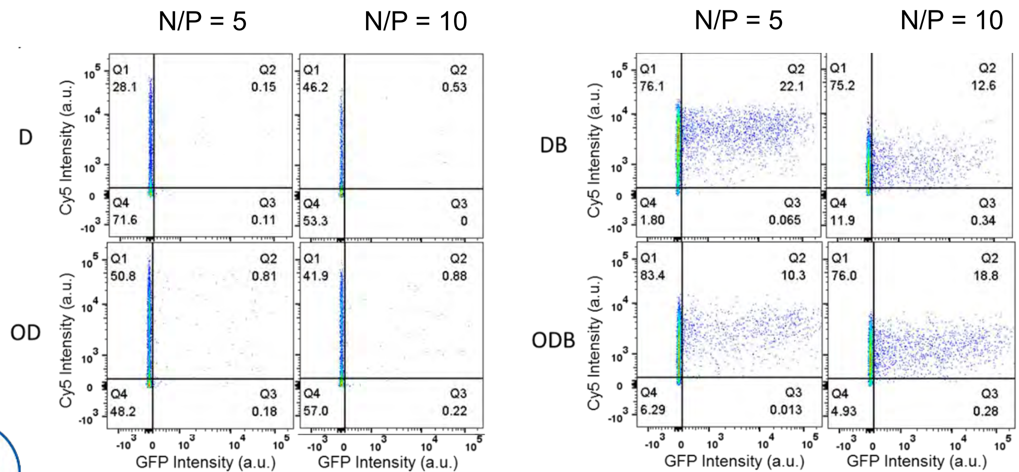
III. Endosomal vesicle escape

IV. DNA expression



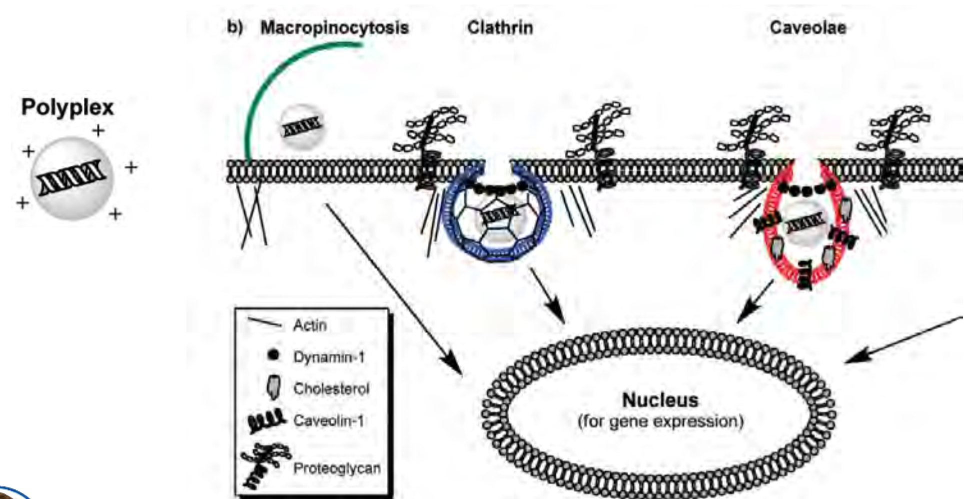
Flow cytometry: micelles enter cells more effectively

~ 35-60% of the cells internalized polyplexes >90% of the cells internalized micelleplexes



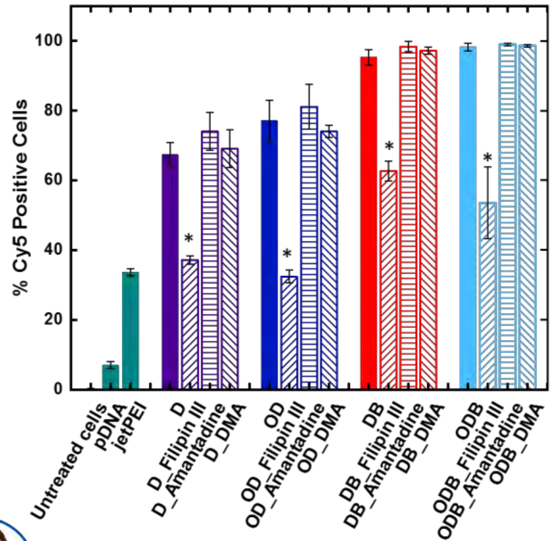
Z. Tan, Y. Jiang, W. Zhang, L. Karls, T. L. Lodge, T. M. Reineke, *J. Am. Chem. Soc.* **2019** 141, 15804–15817 63

Endocytosis pathways of bioconjugates



P. McLendon, K. Fichter, T. M. Reineke, *Mol. Pharmaceutics* **2010**, *7* (3), 738–750 64

All complexes enter cells mainly through caveloae



Caveolae knockdown with fillipin III suggests it is the major entry pathway for all bioconjugate architectures

- Solid bar – no inhibition drugs
- Filipin III – inhibit caveolae pathway
- Amantadine – inhibit clathrin pathway
- (5-(N,Ndimethyl)amiloride hydrochloride) (DMA) – inhibit macropiocytosis pathway

Cellular delivery performance

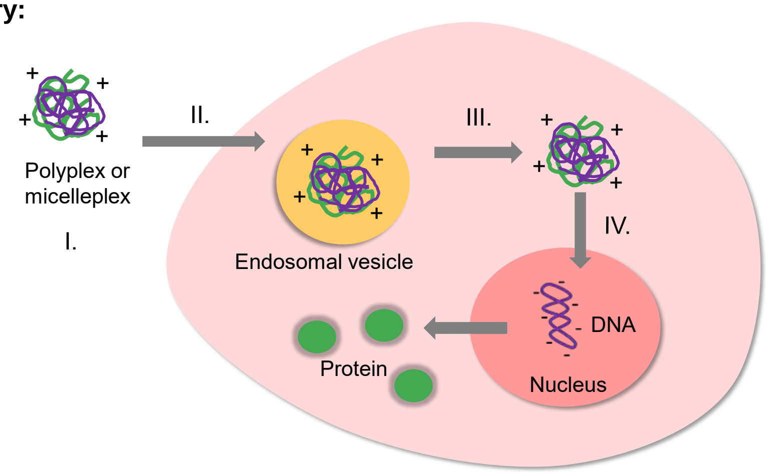
Key factors for successful delivery:

I. Complex formation and colloidal stability

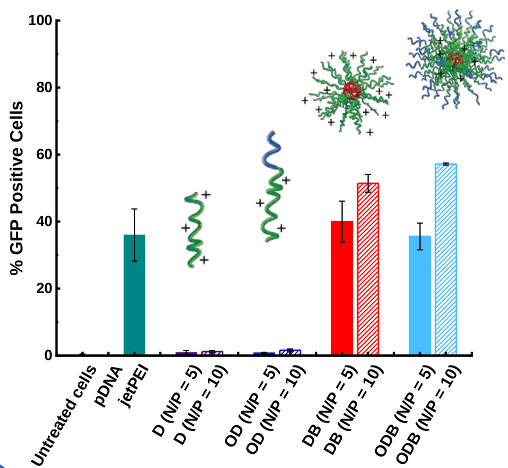
II. Cellular entry efficiency and mechanisms

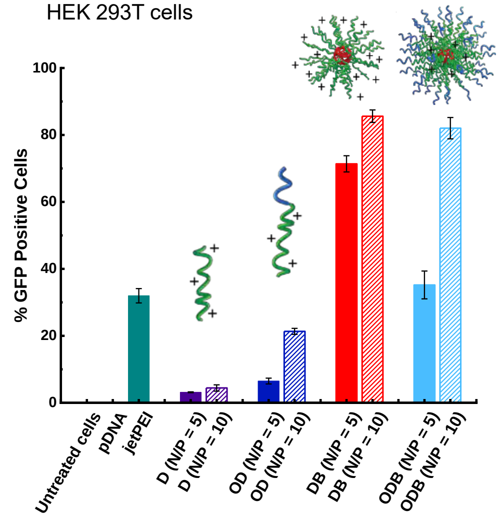
III. Endosomal vesicle escape

IV. DNA expression



Green fluorescent protein (GFP) expression

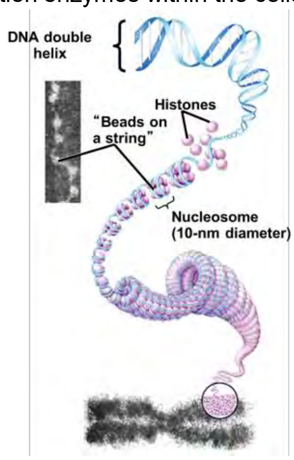


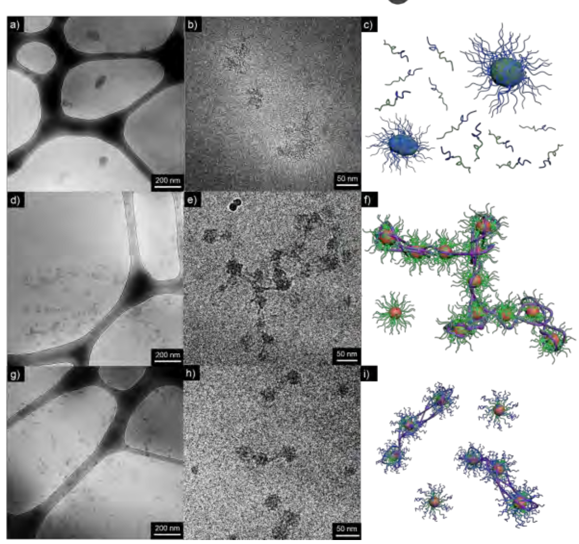


N/P = polycation amine groups/DNA phosphate groups

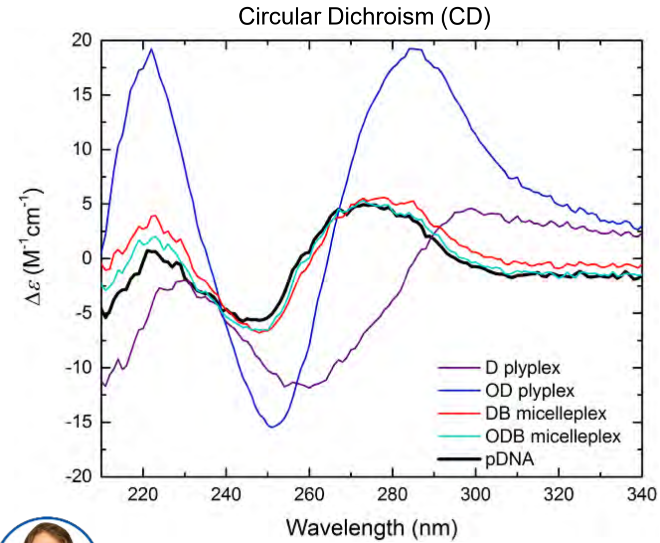
Bioconjugation motif affects performance

- Bioconjugation motif with micelleplexes is reminiscent DNA compaction in chromatin
- may enable more accessibility of the DNA payload to transcription enzymes within the cells





Bioconjugate architecture affects DNA secondary structure



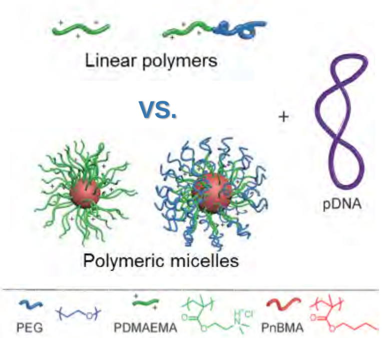
- D polyplex – change of tilt angle between base pairs and helix axis caused by direct interaction between polymer and DNA bases
- OD polyplex - higher level of DNA helicity
- Micelleplex - DNA secondary structure unchanged

➢ **Linear polymers bind to DNA stronger than micelles, which may have challenges to release and expression.**

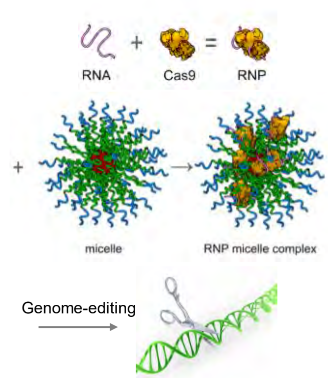
Z. Tan, Y. Jiang, W. Zhang, L. Karls, T. L. Lodge, T. M. Reineke, *J. Am. Chem. Soc.* **2019** 141, 15804–15817

Polymer design and bioconjugate assembly

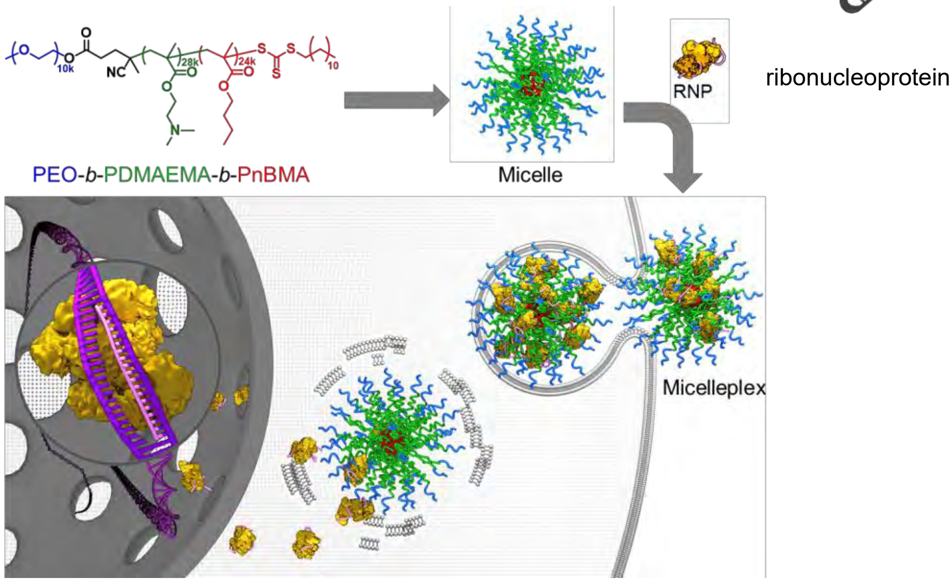
Linear polymers vs micelles for DNA delivery: Influence of polycation architecture



Micelles as CRISPR-Cas9 ribonucleoprotein delivery vehicle: Towards effective genome-editing



Bioconjugation with proteins: gene editing



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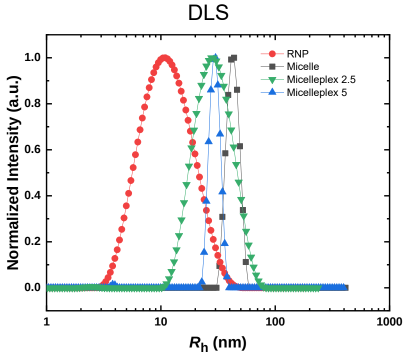
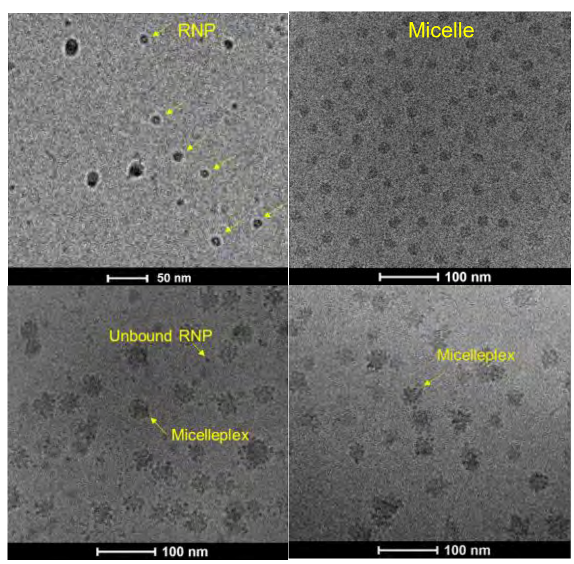
ODB(10) micelle and bioconjugate size in PBS

DLS



Cryo-TEM



- RNP & micelle: isolated single particles
- Micelle: only core can be visualized under cryo-TEM, corona not visible due to low electron density
- Micelleplex shows proteins and SLS reveals 14 proteins conjugated per micelle

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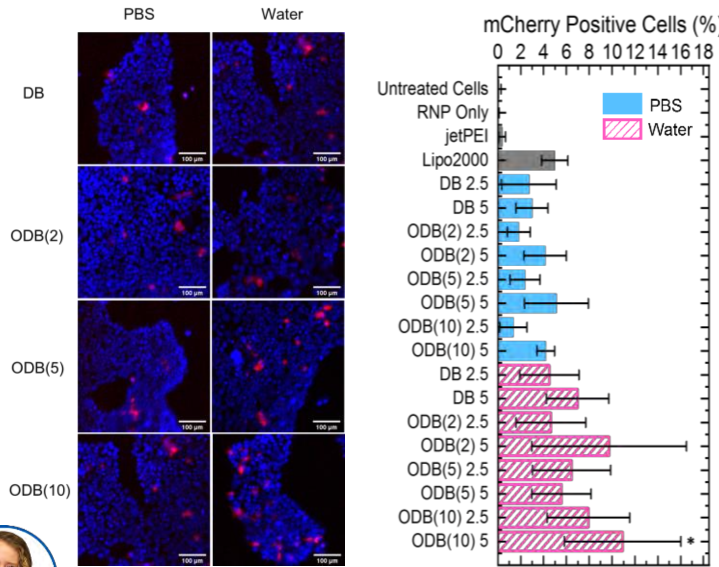
Parallel screening of gene editing efficiency



polymer:RNP molar ratio = 2.5 or 5
engineered HEK-293 cells

- All micelleplexes formulations exhibited similar gene editing efficiency regardless of micelle architecture
- Editing efficiency: bioconjugate formulation in water > in PBS
- ODB(10) variant promoted statistically higher mCherry expression compared to

* Statistically significance compared to Lipo2000 ($p < 0.05$)

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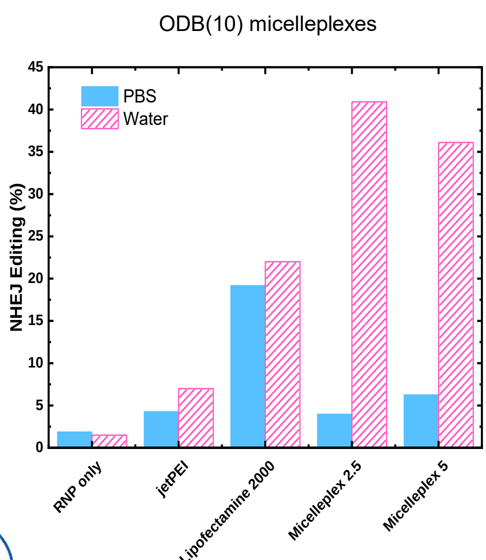
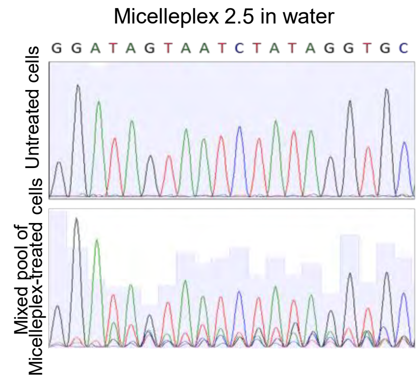
Gene editing quantified by Sanger sequencing



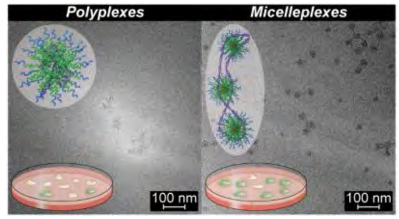


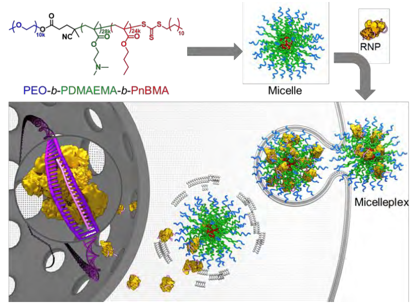
- Formulation of bioconjugates in water promotes larger complexes that contact cells faster and result in higher editing efficiency

Z. Tan, Y. Jiang, W. Zhang, L. Karls, T. L. Lodge, T. M. Reineke, *Macromolecules* **2019,** 52, 8197–8206

Conclusion

- Polymers and their assemblies have been designed as affordable and safe bioconjugates for nucleic acids and proteins
- With all chemistry consistent, micelleplexes outperform analogous polyplexes with linear polymers due to optimal packaging
- The first example of using cationic micelles as CRISPR/Cas9 ribonucleoprotein vehicles
- Polymers and their assemblies offer a tailorable scaffold for multiple bioconjugation purposes





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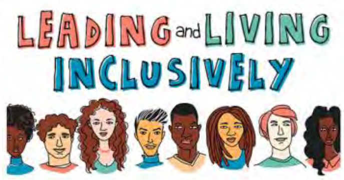

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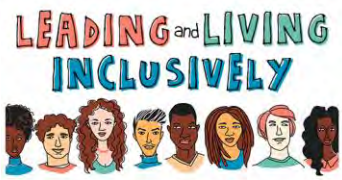

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