A timely perspective on

PEGYLATED LIPID NANOPARTICLES : IMMUNOLOGICAL SAFETY AND EFFICIENCY

Rumiana Tenchov, Janet Sasso, Qiongqiong Angela Zhou

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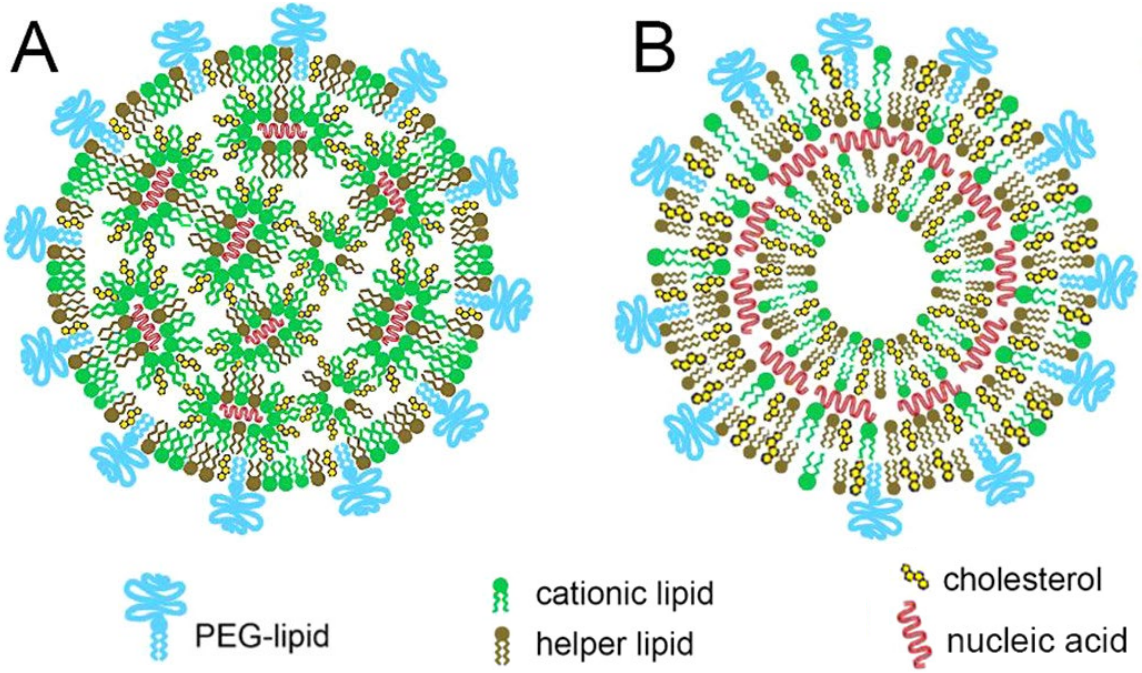
PEG modification is a widely used strategy in drug delivery

From COVID vaccines to therapeutics



PEG: polyethylene glycol invented to conjugate protein drugs

The benefits of PEGylation:

- Reduce ER clearance of drugs
- Prolong drug circulation time
- Improve pharmacokinetics
- Enhance drug efficacy

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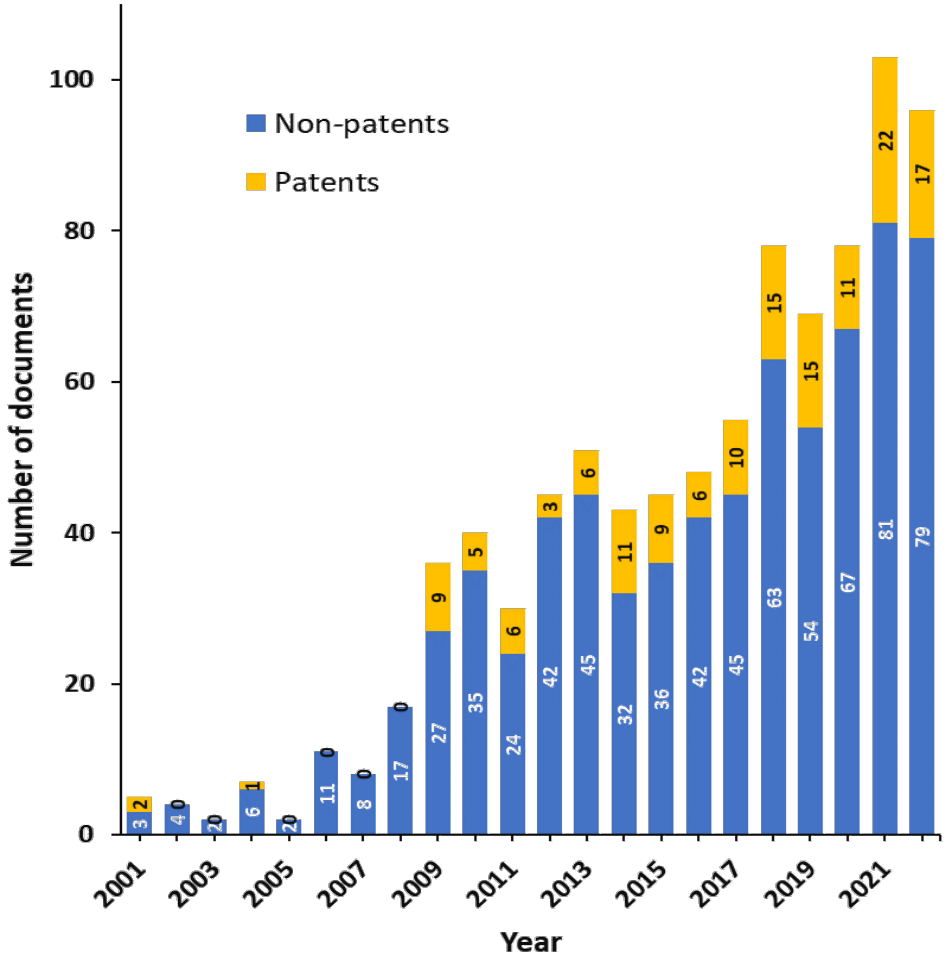
Publication trends of PEG-lipid related adverse immunogenicity

anti-PEG antibodies generation



Reported existence of **anti-PEG antibodies in 25% in healthy blood donors**.
Cancer. 2007;110(1):103–111

Reported **anti-PEG antibodies in about 42% in patients** with no history of treatment with PEGylated products.
Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2015;7 (5):655–677.

US Food and Drug Administration (FDA): Calling for measurement of anti-PEG antibody responses in new drugs that contain PEG molecules

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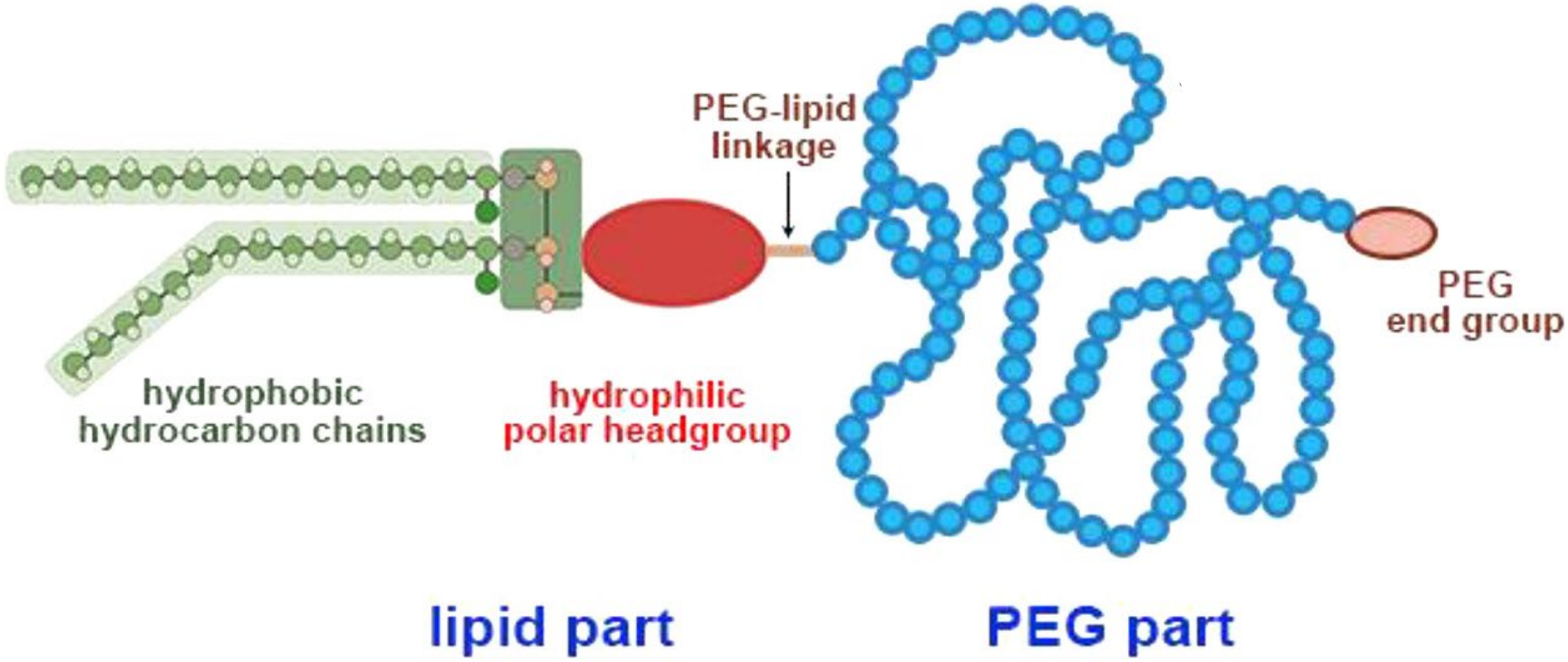
PEG-lipid

Both lipid and PEG parts have an impact on immunogenicity and efficiency



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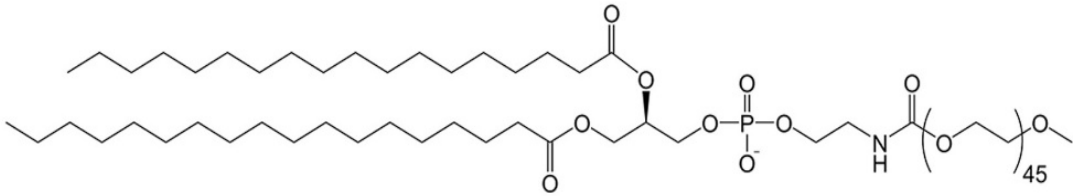
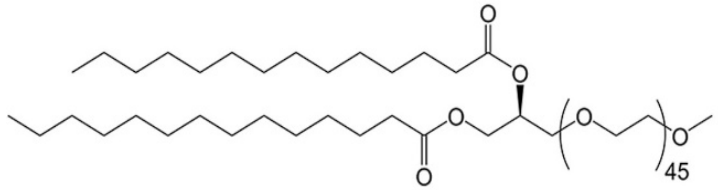
Example PEG-lipids in drugs

PEG2000 is most widely adopted

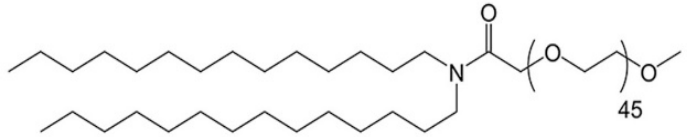
DSPE-mPEG2000 (Doxil)



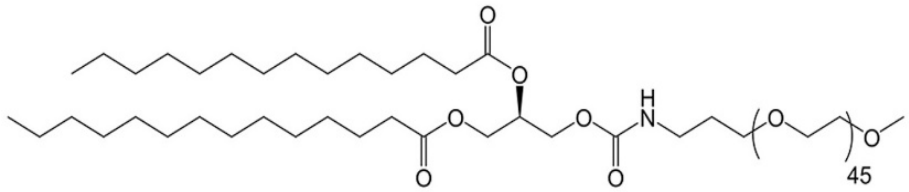
DMG-mPEG2000 (mRNA-1273, Moderna)



ALC-0159 (BNT 162b2, Pfizer / BioNTech)



DMG-C-mPEG2000 (Onpattro)



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PEG length and immunogenicity

Longer PEG more likely induces anaphylaxis

- A higher PEG length → hydrophilicity/flexibility
- 20-50K Da → low molar mass drugs (siRNA, small molecules)
- 1-5K Da → large molecules (antibodies, LNPs)
- Higher MW of PEG → higher chance of induce anaphylaxis
- PEG2000-conjugate lipids are optimal for LNPs. Such lipids have been used in the recent mRNA COVID-19 vaccines.

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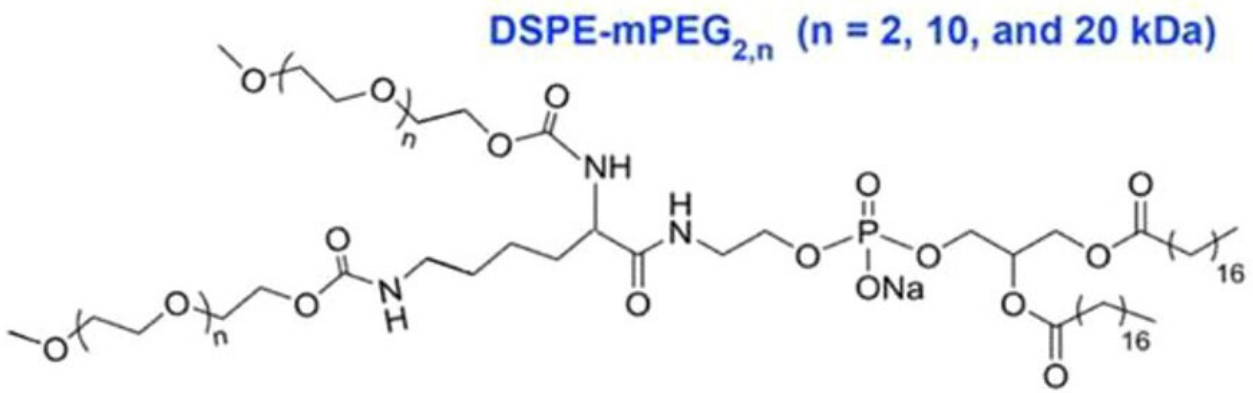
PEG architecture and immunogenicity

Linear vs. branched

- Branched DSPE-mPEG less likely provoke accelerated blood clearance (ABC) than unbranched
- Branched $(mPEG114)_2$–DSPE lipid conjugate has been reported to confer the highest stealth properties to LNPs



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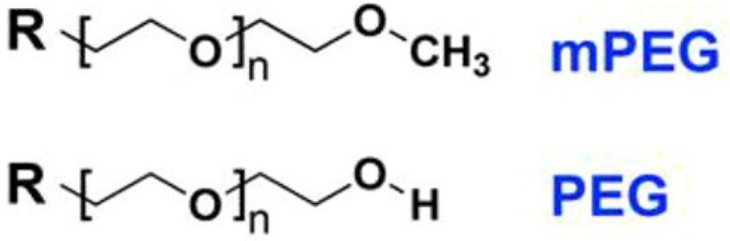
PEG terminal group and immunogenicity

- methoxy (OCH3)-PEG
- amino (NH2)-PEG
- carboxyl (COOH)-PEG
- hydroxy (OH)-PEG: less immunogenic, but rapid clearance
- with respect to anti-PEG IgM production leading to ABC, the PEG terminal groups arrange: $OH < NH_2 < COOH < OCH_3$



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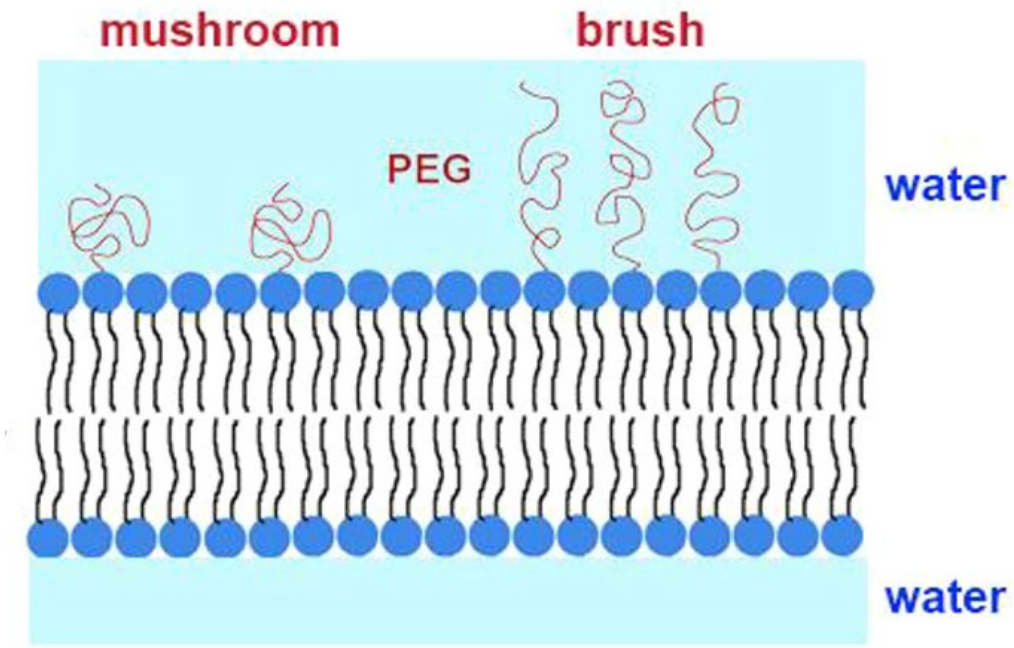
PEG density and immunogenicity

Density (percentage in LNPs)

- Influenced by chain length, molecular weight, concentration
- Low density → mushroom
- High density → brush → less protein adsorption



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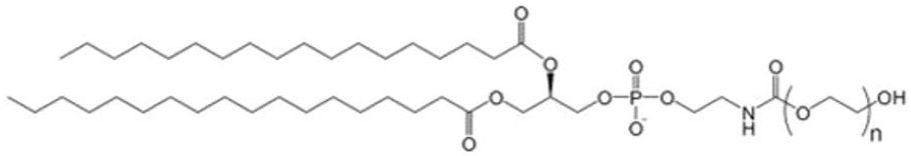
Lipid part

Lipid chain length, lipid headgroup and charge, lipid–PEG linkage

structural parameters/factors	immune-mediated adverse effects/safety/activity aspects
lipid hydrocarbon chains: saturated vs. unsaturated	– Unsaturated phospholipids have higher chances of ABC than saturated phospholipids
lipid anchor: PE vs. diglyceride vs. sterol	– Cholesterol–PEG conjugates have a longer residence time in circulation and a higher systemic bioavailability
lipid hydrocarbon chain length	– Shorter chains (14C) are better than longer (18C) with respect to safety and efficiency
	– Rapid-shedding PEG–lipid (shorter hydrocarbon chains, DMG–PEG) produce a lower amount of anti-PEG IgM than do slower-shedding PEG–lipid (longer hydrocarbon chains, DSG–PEG)
	– For liposomes comprising PEG–ceramide conjugates with C8, C14, C20, and C24 acyl chains, the shorter chain conjugates undergo rapid release from the LNPs after iv administration, while the longer chain conjugates exhibit stronger anchoring in the lipid bilayers
	– Replacing slow-shedding long-chain PEG–lipids (PEG–CerC_{20}) with rapid-shedding short-chain PEG–lipids (PEG–CerC_{14}) abolishes the immune response to PEGylated liposomes
lipid headgroup charge	– The anionic charge at the phosphate group of phospholipid conjugates plays a significant role in the complement activation and anaphylatoxin production; elimination of the negative charge by methylation prevents complement activation
	– Negatively charged PEG lipid with C18-hydrocarbon chains stably associates in lipid particles, while neutral C14 PEG lipid spontaneously shreds out from LNPs
	– Neutral PEGylated liposomes result in accelerated clearance as compared to charged cationic anionic liposomes
hydrocarbon chains–polar headgroup linkage	– Lipid linkage is important for LNP performance: if the ester linkage is replaced by a carbamate one, unstable vesicles are formed
lipid–PEG linkage	– Cleavable PEG–lipid ester linkages significantly attenuate or eliminate the occurrence of the ABC phenomenon

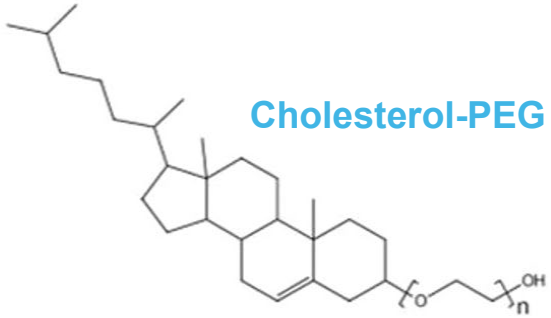
Exemplary PEG–lipid types

DSPE-PEG



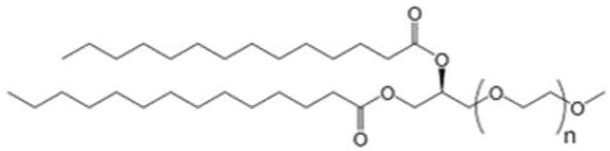
Phospholipid–PEG

Cholesterol-PEG



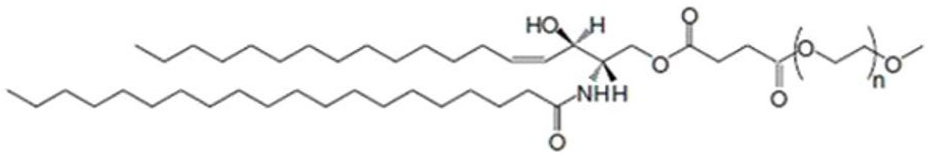
Sterol–PEG

DMG-mPEG



Diglyceride–PEG

$CerC_{20}$-PEG



Ceramide–PEG

Publication frequencies of PEG-lipids

PEG-Lipid	Common name	CAS Registry Number	All documents #	Patents #
PEG-phosphatidylethanolamines (PEG-PE)				
Dilauroyl (12:0/12:0) PE-PEG	DLPE-PEG	2055341-27-8	4	4
Dimyristoyl (14:0/14:0) PE-PEG	DMPE-PEG	211567-66-7; 211733-74-3	107	53
Dipalmitoyl (16:0/16:0) PE-PEG	DPPE-PEG	145035-97-8; 170931-03-0	267	142
Distearoyl (18:0/18:0) PE-PEG	DSPE-PEG	145035-96-7; 170931-04-1	1841	799
Oleoyl-Palmitoyl (18:1c9/16:0) PE-PEG	OPPE-PEG	170127-34-1	6	5
Dioleoyl (18:1c9/18:1c9) PE-PEG	DOPE-PEG	145035-95-6; 262601-19-4	116	60
Dilinoleoyl (18:2c9,12/18:2c9,12) PE-PEG	DLinPE-PEG	736998-47-3	4	4
mPEG-phosphatidylethanolamines (mPEG-PE)				
Dimyristoyl (14:0/14:0) PE-mPEG	DMPE-mPEG	474922-82-2; 261764-82-3	101	65
Dipalmitoyl (16:0/16:0) PE-mPEG	DPPE-mPEG	205494-72-0	91	50
Distearoyl (18:0/18:0) PE-mPEG	DSPE-mPEG	156543-00-9; 247925-28-6; 474922-77-5; 459428-35-4	2015	614
Dioleoyl (18:1c9/18:1c9) PE-mPEG	DOPE-mPEG	226940-29-0	53	26
mPEG-glycerides				
Dimyristoyl (14:0/14:0) glycerol-mPEG	DMG-PEG	160743-62-4; 1397695-86-1	556	429
Dipalmioyl (16:0/16:0) glycerol-mPEG	PDG-PEG	162409-28-1	31	25
Distearoyl (18:0/18:0) glycerol-mPEG	DSG-PEG; Sunbright DSG 2H	308805-39-2; 850628-36-3	76	50
Dioleoyl (18:1c9/18:1c9) glycerol-mPEG	DOG-PEG	160743-61-3	6	5

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Publication frequencies of PEG-lipids

PEG-Lipid	Common name	CAS Registry Number	All documents #	Patents #
amino-mPEGs				
Dilauroyl (12:0/12:0) amino-PEG		1849616-44-9	2	2
Lauroyl-Myristoyl (12:0/14:0) amino-PEG		1849616-45-0	1	1
Dimyristoyl (14:0/14:0) amino-PEG	ALC-0159	1849616-42-7	27	26
Dipalmitoyl (16:0/16:0) amino-PEG		1849616-43-8	2	2
Distearoyl (18:0/18:0) amino-PEG		741737-56-4	3	2
Chol-PEG				
Cholesterol-PEG	PEG-cholesterol	27321-96-6	626	460
Cholesterol-mPEG	mPEG-cholesterol	99559-58-7	27	14
Cholesterol-PEG-amine	Chol-PEG-NH_2	444045-24-3	11	3
PEG-cholesteryl carbonate	PEG-CHMC	146185-41-3	5	1
mPEG-ceramides				
N-octanoyl-sphingosine-mPEG	C8 PEG Ceramide	212116-76-2	31	20
N-palmitoyl-sphingosine-mPEG	C16 PEG Ceramide	212116-78-4	85	38

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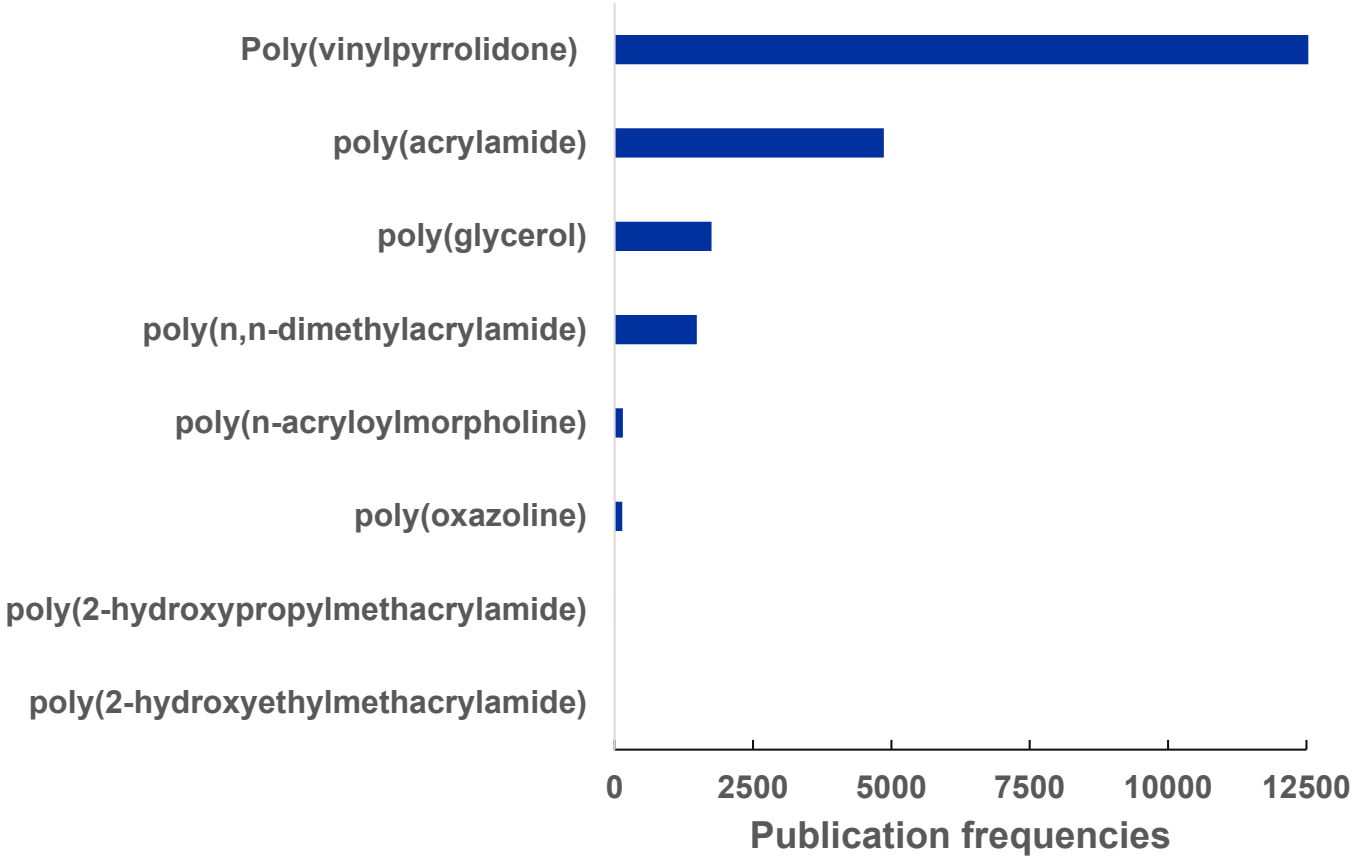
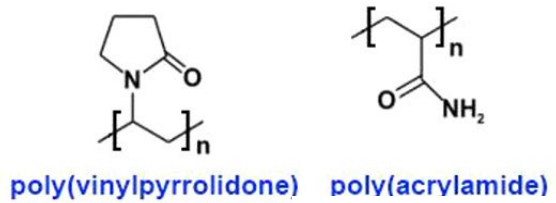
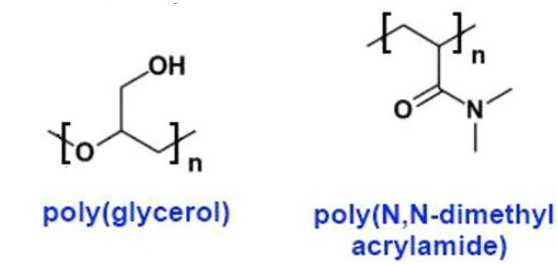
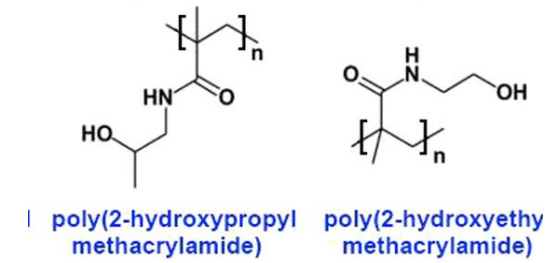
Alternative polymeric modifications

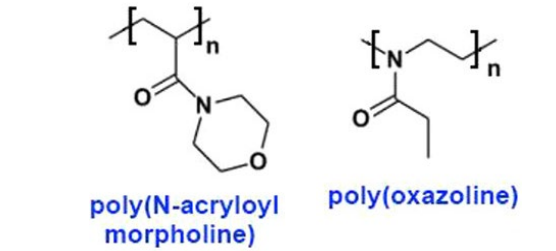
Poly(vinylpyrrolidone) is most extensively studied











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Approved PEG-lipid related drugs

From small molecules to biologics

Trade name	Approval	Active ingredient	Lipid composition	Indication for use	Immuno-induced adverse effects
Doxil/Caelyx	1995, 1996	Doxorubicin	HSPC:Chol:PEG2000–DSPE (56:39:5)	ovarian, breast cancer, Kaposi's sarcoma	ABC, CARPA
ThermoDox	2014	Doxorubicin	DPPC, MSPC, PEG2000–DSPE	hepatocellular carcinoma	CARPA
Onivyde	2015	Irinotecan	DSPC:mPEG–2000:DSPE (3:2:0.015)	metastatic pancreatic adenocarcinoma	hypersensitivity, anaphylaxis
Onpattro (Patisiran)	2018	RNAi, transthyretin-directed siRNA	DLin-MC3-DMA, PEG2000-C-DMG, DSPC, Chol	hATTR amyloidosis	CARPA
Lipoplatin	2018	cisplatin	HSPC/DPPG/DSPE-mPEG2000	NSCLC, breast tumor, gastric tumor	N/A
BNT162b2 (Comirnaty; tozinameran)	2021	mRNA	ALC-0315:ALC-0159:Chol:DSPC (46.3:1.6:42.7:9.4)	COVID-19 vaccine	anaphylaxis
mRNA-1273 (Spikevax)	2021	mRNA	SM-102:PEG2000-DMG:Chol:DSPC (50:1.5:38.5:10)	COVID-19 vaccine	hypersensitivity, anaphylaxis

Clinical trials of LNP-related drugs

Mostly cancer drugs and COVID vaccines

Clinical trial identifier	Interventions	Conditions
NCT03483038	PEGylated Liposomal Irinotecan (Onivyde)/FOLFOX regimen	pancreatic cancer
NCT05029999	PEGylated Liposomal Doxorubicin (Doxil)	breast cancer
NCT05388487	PEGylated Liposomal All-Trans Retinoic Acid (HF1K16)	solid tumor
NCT01210768	PEGylated Liposomal Doxorubicin/Cyclophosphamide	breast cancer
NCT02839707	PEGylated Liposomal Doxorubicin/Atezolizumab/Bevacizumab	ovarian, fallopian tube, and peritoneal cancer
NCT03088813	PEGylated Liposomal Irinotecan (Onivyde)/Topotecan	lung cancer
NCT04715438	Spikevax	SARS-CoV-2
NCT05618548	Comirnaty/Spikevax	SARS-CoV-2
NCT05000216	Comirnaty/Spikevax	SARS-CoV-2
NCT05077254	Comirnaty	SARS-CoV-2

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Gain insights on the landscape of PEGylated LNPs and more

Peer reviewed journal article
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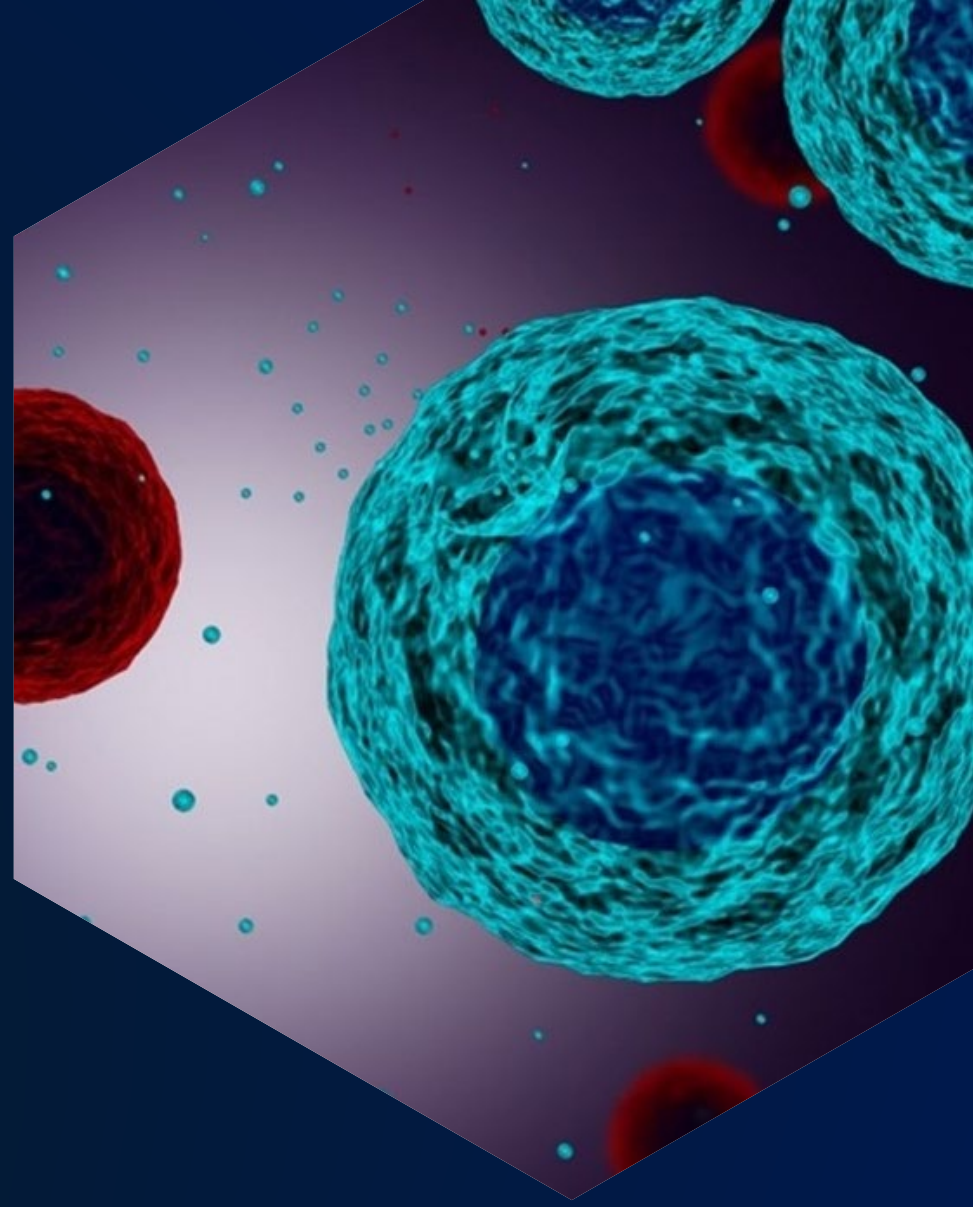

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