学校编码：10384

学号：20520140153967

厦门大学

博 士 学 位 论 文

超高灵敏流式检测技术在脂质纳米药物多参数定量表征中的应用

Multiparameter Quantification of Lipid Nanomedicines by High Sensitivity Flow Cytometry

陈超翔

指导教师：颜晓梅

专业名称：化学生物学

答辩日期：2017年9月

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摘 要

纳米药物因其在靶向给药、药物缓控释、提高难溶性药物与多肽药物的生物利用度、降低药物毒副作用等方面显示出的良好应用前景，而成为21 世纪生物医学领域的重要发展方向。纳米药物的载体包括高分子纳米颗粒、脂质纳米颗粒、无机纳米颗粒、病毒类纳米颗粒等，其中以磷脂为主要骨架基元的脂质纳米药物（主要包括脂质体、实心脂质纳米粒、磷脂胶束和细胞分泌囊泡等）具有良好的稳定性、优异的载药量和生物相容性，是目前临床上最受认可的载药系统。

为了加快脂质纳米药物的研发进程并保障临床应用的药效，必须对纳米颗粒的粒径及其分布、颗粒浓度、药物包封率、载药量、表面配体数量及其分布等物理、化学性状进行快速、准确的表征。由于脂质纳米药物具有高度的异质性，唯有在单颗粒水平对纳米药物进行快速地逐一分析，方可揭示因集权平均而被掩盖的个体差异，获得具有统计代表意义的性状分布特征。然而，脂质纳米颗粒粒径微小、结构脆弱，使得在单颗粒水平对其进行多参数定量检测极具挑战性。虽然冷冻透射电镜（cryo-TEM）能够最大程度地维持脂质纳米药物的原貌，清晰地揭示纳米颗粒的粒径及微观结构，但是存在样品制备繁琐、试样固定时纳米颗粒易团聚、测量速度慢、仪器设备极其昂贵等缺陷。而现有的其他商品化单颗粒表征技术在灵敏度、分辨率、分析速度或多参数表征等方面尚难以满足脂质纳米药物的表征需求。因此，发展一种快速、灵敏、高通量的单颗粒水平定量表征技术对于脂质纳米药物的研发和应用至关重要。

流式细胞仪是一种对悬液中的细胞或细胞大小的颗粒进行快速分析或分选的单细胞检测技术，具有快速、多参数、定量检测等优点。然而，传统流式细胞仪由于检测灵敏度的限制，只能检测到粒径大于500 nm 或荧光亮度大于200 个荧光素分子的信号。而纳米药物载体颗粒的粒径一般小于200 nm，且折射率低，使得传统流式细胞仪在脂质纳米药物的检测方面难以发挥作用。结合瑞利散射和鞘流单分子荧光检测技术，我们课题组发展了超高灵敏流式检测技术（high sensitivity flow cytometry， HSFCM），在国际上首次实现了发光能力低于

单分子荧光的单个纳米颗粒散射光信号的直接检测，将二氧化硅纳米颗粒和纳米金的散射光检出下限分别推进到24 nm 和7 nm，荧光信号检测限达到3 个Alexa Fluor 555 荧光分子，灵敏度比传统流式细胞仪提升了3-5 个数量级。HSFCM 通过对单个纳米颗粒散射光强度的直接检测实现颗粒粒径的高分辨表征，每分钟检测速率高达10000 个颗粒，仅需数分钟即可获得透射电镜需要数小时才能得到测试结果。此外，通过对颗粒的荧光信号进行同时检测，可实现单个纳米颗粒生物化学性状的多参数定量表征以及各参数之间的相关性分析。基于HSFCM 优异的分辨率、灵敏度、检测速度及多参数表征的能力，本论文建立了一种在单颗粒水平对多种脂质纳米药物进行快速分析的方法，实现了脂质纳米药物粒径、颗粒浓度、载药量、包封率、表面配体数量及密度的多参数定量表征，为脂质纳米药物的合成优化、质量控制和临床应用提供一种快速、高效、实用的表征方法。本论文主要包括以下几方面内容：

第一章为文献综述。简要介绍纳米药物的研究背景和国际上常用的脂质纳米药物单颗粒表征技术，以及本论文的选题思路和研究内容。

第二章为基于散射光检测的脂质纳米药物粒径表征。通过HSFCM 卓越的灵敏度实现了对阿霉素脂质体散射光信号的测定，并以二氧化硅纳米颗粒作为标准球将散射光强度转换为对应的粒径分布。另外，通过讨论颗粒折射率对粒径表征结果的影响，我们还提出了通过瑞利散射定律对二氧化硅标准球散射光强度进行校正以提高粒径测定结果的准确性。最后，为了实现标准品和待测样折射率的完全匹配，我们制备了四个不同大小的阿霉素脂质体并通过冷冻透射电镜表征其准确粒径，以之作为标准品用于对其他阿霉素脂质体样品粒径的精确测定。

第三章为基于散射光和荧光检测的脂质纳米药物多参数表征。通过HSFCM 的散射光和荧光双参数同时检测能力，实现对了阿霉素脂质体内微弱自发荧光的测定，并据此实现了阿霉素脂质体载药比率、血清稳定性和颗粒浓度的定量表征。此外，分别通过荧光二氧化硅标准球和阿霉素脂质体标准品建立荧光强度与阿霉素含量之间的关系，实现了脂质体载药量的测定。最后，我们根据建立的方法实现了对商品化阿霉素脂质体Doxoves 和gDoxil 以及临床II 期基因纳米药物siRNA 脂质纳米粒的多参数定量表征。

第四章为靶向型脂质体的制备与表征。首先以叶酸脂质体作为模型建立了脂质体表面配体密度的定量表征方法，并以此考察脂质体与细胞结合的最佳配体密度。此外，还分析了不同制备方法及PEG 层厚度对表面配体密度及其分布异质性的影响。最后，还将该方法应用于表征以转铁蛋白和单克隆抗体作为靶向基元的脂质纳米颗粒的表面配体含量，并建立了评估有效配体在脂质体表面总蛋白含量中所占比例的实验方法。

第五章为总结与展望。总结本论文的研究内容，并对今后研究工作进行展望。

关键词：超高灵敏流式检测技术；单颗粒；纳米药物；脂质体；多参数表征

Abstract

The growing interest in the biomedical application of nanomedicine is largely attributable to its unique and appealing features in targeted delivery, controlled release
and improved bioavailability. Currently, nanomedicines can be broadly divided into the
following categories: lipid nanoparticles, polymeric nanoparticles, inorganic nanoparticles and virus nanoparticles. Among them, lipid based nanomedicine such as
liposomes, solid lipid nanoparticles, lipid micelles and extracellular vesicles, which are
characterized by high biological compatibility and substantial drug-loading capability,
are considered the most successful drug delivery systems known to date.
To achieve sufficient quality control and highly-efficient drug delivery, properties such as particle size and size distribution, particle concentration, drug content, fraction
of drug encapsulation and surface ligand density must be precisely characterized.
Because of the complexity and the large intrinsic heterogeneity of lipid nanomedicines
in particle size and composition, rapid and rigorous characterization at the singleparticle
level is of fundamental importance to reveal the individual difference that
would be otherwise averaged out in ensemble measurements. However, it is very challenging to achieve multiparameter and quantitative analysis of lipid nanomedicines
at the single particle level on account of their small sizes and poor structure

stability.

Whereas cryo-TEM can provide valuable size and morphology information with maximum structure preservation of lipid nanomedicines, its routine application is prevented due to the poor accessibility, low throughout and the time-consuming sample
preparation. Other commercialized single-particle techniques also fall short in fulfilling
the requirement for nanomedicine characterization owing to the limited sensitivity and
resolution or the lack of multiparameter analysis ability. Thus, it is of vital importance
to develop a rapid, sensitive and high-throughput quantitative characterization method
at the single particle level for the analysis and development of lipid nanomedicines.

Flow cytometry is a single particle detection technique for rapid, multiparameter and quantitative analysis and sorting of individual cells or cell-size particles in suspension. Due to the limitation of sensitivity, conventional flow cytometer can only
be applied to the analysis of particles with large size or strong fluorescence. However,
it is extremely difficult for conventional flow cytometer to achieve multiparameter analysis of drug-loading lipid nanoparticles smaller than 200 nm. Integrating light scattering with strategies for single-molecule fluorescence detection in a sheathed flow,
our laboratory have developed high sensitivity flow cytometry (HSFCM) that enables
light-scattering detection of single nanoparticles with light-producing power below

the
level of single fluorescent molecules for the first time. The light-scattering based size
detection limits for single nanoparticles are 24 nm and 7 nm in diameter for silica and
gold nanoparticles, respectively. And the fluorescence detection limit is three
fluorescent molecules of alexa fluor 555. By offering high-resolution analysis of single
nanoparticles in liquid suspensions at a throughput of up to 10 000 particles per minute,
HSFCM can reveal the size distribution in minutes which would take hours for cryo-
TEM to accomplish. Moreover, through fluorescence detection, the multiple
biochemical properties of single particles and their correlation can be quantitatively
characterized.
Taking advantage of the superior resolution, sensitivity, detection speed and the
capability of multi-parameter analysis of HSFCM, we develop a rapid characterization
method for lipid nanomedicines in this dissertation, which allows the absolute
quantification of particle size, drug content, fraction of drug encapsulation, particle
concentration and the surface ligand density of liposomal nanomedicines at the
singleparticle
level. This novel method aims to meet the characterization challenge of lipid
nanomedicine and to promote its development. This dissertation consists of the
following sections:
In chapter one, the research background of nanomedicine and the single particle
detection techniques for nanomedicine analysis are briefly introduced.

Chapter two introduces the side scattering based size measurement of lipid nanomedicines by HSFCM. Using silica nanoparticles with various size as the standards, we successfully characterized the size distribution of doxorubicin-loaded liposomes. Furthermore, we discuss the influence of the particle refractive index on the
light-scattering based detection of liposomal nanoparticles. We propose that a calibration of scattering intensity should be applied via Rayleigh scattering theory when
sizing nanoparticles with refractive index different from the standards. At last, to achieve a perfect match of particle refractive index, we developed a strategy by using
well prepared and size characterized doxorubicin-loaded liposomes as standards for the
measurement of commercial nanomedicine products.
Chapter three presents the side scattering and fluorescence dual-parameter detection of lipid nanomedicines. By means of that, drug encapsulation fraction, serum
stability and particle concentration of doxorubicin encapsulated liposomes could be
characterized. Furthermore, by using fluorescent silica nanoparticles with various molecules of equivalent soluble fluorochrome (MESF) or the well-prepared doxorubicin-loaded liposomes as standards respectively, we revealed the drug content
distribution of doxorubicin-loaded liposomes. Applications of this analysis method for
the multiparameter characterization of commercial doxorubicin-loaded liposomes (Doxoves and gDoxil) and siRNA encapsulated lipid nanoparticles were also demonstrated.

Chapter four describes the preparation and multiparameter characterization of targeted liposomes. Firstly, using folate coupled liposomes as a model, we develop a
quantitative characterization method for the surface ligand density and studied its biological impact on the cell binding ability of liposomes. Besides, the influence of preparation methods and PEG length on the ligand density distribution and heterogeneity is also elucidated. Finally, the method is applied to the transferrin or antibody coupled liposomes, and a novel evaluation method for the proportion of available ligand number in total protein content is established.
In chapter five, the work of this thesis is summarized and the future prospect in single lipid nanoparticle analysis is discussed.

Keywords: high-sensitivity flow cytometry; single particle; nanomedicine; liposome; multiparameter characterization

参考资料

第一章

[1] Koo O. M.; Rubinstein I.; Onyuksel H., Role of nanotechnology in targeted drug delivery and imaging: A concise review[J]. Nanomedicine-Nanotechnology Biology and Medicine 2005, 1(3): 193-212.

[2] Kawasaki E. S.; Player A., Nanotechnology, nanomedicine, and the development of new, effective therapies for cancer[J]. Nanomedicine 2005, 1(2): 101-109.

[3] Kendall M.; Lynch I., Long-term monitoring for nanomedicine implants and drugs[J]. Nature Nanotechnology 2016, 11(3): 206-210.

[4] Wagner V.; Dullaart A.; Bock A. K., et al., The emerging nanomedicine landscape[J]. Nature Biotechnology 2006, 24(10): 1211-1217.

[5] Shi J.; Kantoff P. W.; Wooster R., et al., Cancer nanomedicine: Progress, challenges and opportunities[J]. Nature Reviews Cancer 2017, 17(1): 20-37.

[6] Wicki A.; Witzigmann D.; Balasubramanian V., et al., Nanomedicine in cancer therapy: Challenges, opportunities, and clinical applications[J]. Journal of Controlled Release 2015, 200(1): 138-157.

[7] Doane T. L.; Burda C., The unique role of nanoparticles in nanomedicine: Imaging, drug delivery and therapy[J]. Chemical Society Reviews 2012, 41(7): 2885-2911.

[8] Yoo J. W.; Irvine D. J.; Discher D. E., et al., Bio-inspired, bioengineered and biomimetic drug delivery carriers[J]. Nature Reviews Drug Discovery 2011, 10(7): 521-535.

[9] Tiwari G.; Tiwari R.; Sriwastawa B., et al., Drug delivery systems: An updated review[J]. International Journal of Pharmaceutical Investigation 2012, 2(1): 2-11.

[10] Duncan R.; Vicent M. J., Polymer therapeutics-prospects for 21st century: The end of the beginning[J]. Advanced Drug Delivery Reviews 2013, 65(1): 60-70.

[11] Allen T. M.; Cullis P. R., Liposomal drug delivery systems: From concept to clinical applications[J]. Advanced Drug Delivery Reviews 2013, 65(1): 36-48.

[12] Boisselier E.; Astruc D., Gold nanoparticles in nanomedicine: Preparations, imaging, diagnostics, therapies and toxicity[J]. Chemical Society Reviews 2009, 38(6): 1759-1782.

[13] Garcia J.; Tang T.; Louie A. Y., Nanoparticle-based multimodal PET/MRI probes[J]. Nanomedicine (Lond) 2015, 10(8): 1343-1359.

[14] Huynh N. T.; Passirani C.; Saulnier P., et al., Lipid nanocapsules: A new platform for nanomedicine[J]. International Journal of Pharmaceutics 2009, 379(2): 201-209.

[15] Namiki Y.; Fuchigami T.; Tada N., et al., Nanomedicine for cancer: Lipid-based nanostructures for drug delivery and monitoring[J]. Accounts of Chemical Research 2011, 44(10): 1080-1093.

[16] Bangham A. D.; Horne R. W., Negative staining of phospholipids and their structural modification by surface-active agents as observed in the electron microscope[J]. Journal of Molecular Biology 1964, 8(1): 660-668.

[17] Akbarzadeh A.; Rezaei-Sadabady R.; Davaran S., et al., Liposome: Classification, preparation, and applications[J]. Nanoscale Research Letters 2013, 8(1): 102-110.

[18] Matsumura Y.; Maeda H., A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs[J]. Cancer Research 1986, 46(12): 6387-6392.

[19] Maeda H., Toward a full understanding of the EPR effect in primary and metastatic tumors as well as issues related to its heterogeneity[J]. Advanced Drug Delivery Reviews 2015, 91(1): 3-6.

[20] Gerlowski L. E.; Jain R. K., Microvascular permeability of normal and neoplastic tissues[J]. Microvascular Research 1986, 31(3): 288-305.

[21] Barenholz Y., Doxil(R)--the first FDA-approved nano-drug: Lessons learned[J]. Journal of Controlled Release 2012, 160(2): 117-134.

[22] Milla P.; Dosio F.; Cattel L., Pegylation of proteins and liposomes: A powerful and flexible strategy to improve the drug delivery[J]. Current Drug Metabolism 2012, 13(1): 105-119.
[23] Leserman L. D.; Barbet J.; Kourilsky F., et al., Targeting to cells of fluorescent liposomes covalently coupled with monoclonal antibody or protein A[J]. Nature 1980, 288(5791): 602-604.
[24] Zhang S.; Zhao Y., Controlled release from cleavable polymerized liposomes upon redox and pH stimulation[J]. Bioconjugate Chemistry 2011, 22(4): 523-528.
[25] Shum P.; Kim J. M.; Thompson D. H., Phototriggering of liposomal drug delivery systems[J]. Advanced Drug Delivery Reviews 2001, 53(3): 273-284.
[26] Park S. M.; Kim M. S.; Park S. J., et al., Novel temperature-triggered liposome with high stability: Formulation, in vitro evaluation, and in vivo study combined with high-intensity focused ultrasound (HIFU)[J]. Journal of Controlled Release 2013, 170(3): 373-379.
[27] Pattni B. S.; Chupin V. V.; Torchilin V. P., New developments in liposomal drug delivery[J]. Chemical Reviews 2015, 115(19): 10938-10966.
[28] Lukyanov A. N.; Torchilin V. P., Micelles from lipid derivatives of water-soluble polymers as delivery systems for poorly soluble drugs[J]. Advanced Drug Delivery Reviews 2004, 56(9): 1273-1289.
[29] Gill K. K.; Kaddoumi A.; Nazzal S., PEG-lipid micelles as drug carriers: Physiochemical attributes, formulation principles and biological implication[J]. Journal of Drug Targeting 2015, 23(3): 222-231.
[30] Gujrati V.; Kim S.; Kim S. H., et al., Bioengineered bacterial outer membrane vesicles as cell-specific drug-delivery vehicles for cancer therapy[J]. ACS Nano 2014, 8(2): 1525-1537.
[31] van der Meel R.; Fens M. H.; Vader P., et al., Extracellular vesicles as drug delivery systems: Lessons from the liposome field[J]. Journal of Controlled Release 2014, 195(1): 72-85.
[32] Villa C. H.; Anselmo A. C.; Mitragotri S., et al., Red blood cells: Supercarriers for drugs, biologicals, and nanoparticles and inspiration for advanced delivery systems[J]. Advanced Drug Delivery Reviews 2016, 106(Part A): 88-103.
[33] Fang R. H.; Hu C. M.; Luk B. T., et al., Cancer cell membrane-coated nanoparticles for anticancer vaccination and drug delivery[J]. Nano Letters 2014, 14(4): 2181-2188.
[34] Caster J. M.; Patel A. N.; Zhang T., et al., Investigational nanomedicines in 2016: A review of nanotherapeutics currently undergoing clinical trials[J]. Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology 2017, 9(1): 1-18.
[35] Etheridge M. L.; Campbell S. A.; Erdman A. G., et al., The big picture on nanomedicine: The state of investigational and approved nanomedicine products[J]. Nanomedicine 2013, 9(1): 1-14.
[36] Blanco E.; Shen H.; Ferrari M., Principles of nanoparticle design for overcoming biological barriers to drug delivery[J]. Nature Biotechnology 2015, 33(9): 941-951.
[37] Sun Q. H.; Zhou Z. X.; Qiu N. S., et al., Rational design of cancer nanomedicine: Nanoproperty integration and synchronization[J]. Advanced Materials 2017, 29(14): 1-18.
[38] Richter A. G.; Dergunov S. A.; Ganus B., et al., Scattering studies of hydrophobic monomers in liposomal bilayers: An expanding shell model of monomer distribution[J]. Langmuir 2011, 27(7): 3792-3797.
[39] Bouwstra J. A.; Gooris G. S.; Bras W., et al., Small angle X-ray scattering: Possibilities and limitations in characterization of vesicles[J]. Chemistry and Physics of Lipids 1993, 64(1-3): 83-98.
[40] Zhong Z.; Ji Q.; Zhang J. A., Analysis of cationic liposomes by reversed-phase HPLC with evaporative light-scattering detection[J]. Journal of Pharmaceutical and Biomedical Analysis 2010, 51(4): 947-951.
[41] Holzer M.; Barnert S.; Momm J., et al., Preparative size exclusion chromatography combined with detergent removal as a versatile tool to prepare unilamellar and spherical liposomes of highly uniform size distribution[J]. Journal of Chromatography A 2009, 1216(31): 5838-5848.
[42] Hupfeld S.; Moen H. H.; Ausbacher D., et al., Liposome fractionation and size analysis by asymmetrical flow field-flow fractionation/multi-angle light scattering: Influence of ionic strength and osmotic pressure of the carrier liquid[J]. Chemistry and Physics of Lipids 2010, 163(2): 141-147.
[43] Baruah B.; Surin A., Interaction of liposome-encapsulated cisplatin with biomolecules[J]. Journal of

Biological Inorganic Chemistry 2012, 17(6): 899-910.
[44] Adrian M.; Dubochet J.; Lepault J., et al., Cryo-electron microscopy of viruses[J]. Nature 1984, 308(5954): 32-36.
[45] Severs N. J., Freeze-fracture electron microscopy[J]. Nature Protocols 2007, 2(3): 547-576.
[46] Zasadzinski J. A., Transmission electron microscopy observations of sonication-induced changes in liposome structure[J]. Biophysical Journal 1986, 49(6): 1119-1130.
[47] Tarahovsky Y. S.; Yagolnik E. A.; Muzafarov E. N., et al., Calcium-dependent aggregation and fusion of phosphatidylcholine liposomes induced by complexes of flavonoids with divalent iron[J]. Biochimica et Biophysica Acta 2012, 1818(3): 695-702.
[48] Bibi S.; Kaur R.; Henriksen-Lacey M., et al., Microscopy imaging of liposomes: From coverslips to environmental SEM[J]. International Journal of Pharmaceutics 2011, 417(1-2): 138-150.
[49] Almgren M.; Edwards K.; Karlsson G., Cryo transmission electron microscopy of liposomes and related structures[J]. Colloids and Surfaces A-Physicochemical and Engineering Aspects 2000, 174(1-2): 3-21.
[50] Frederik P. M.; Hubert D. H. W., Cryoelectron microscopy of liposomes[J]. Methods in Enzymology 2005, 391(1): 431-448.
[51] Lepault J.; Pattus F.; Martin N., Cryo-electron microscopy of artificial biological-membranes[J]. Biochimica et Biophysica Acta 1985, 820(2): 315-318.
[52] Egelhaaf S. U.; Wehrli E.; Muller M., et al., Determination of the size distribution of lecithin liposomes: A comparative study using freeze fracture, cryoelectron microscopy and dynamic light scattering[J]. Journal of Microscopy 1996, 184(1): 214-228.
[53] Edwards K.; Johnsson M.; Karlsson G., et al., Effect of polyethyleneglycol-phospholipids on aggregate structure in preparations of small unilamellar liposomes[J]. Biophysical Journal 1997, 73(1): 258-266.
[54] Wibroe P. P.; Ahmadvand D.; Oghabian M. A., et al., An integrated assessment of morphology, size, and complement activation of the pegylated liposomal doxorubicin products Doxil (R), Caelyx (R), DOXOrubicin, and SinaDoxosome[J]. Journal of Controlled Release 2016, 221(1): 1-8.
[55] Zidovska A.; Ewert K. K.; Quispe J., et al., The effect of salt and pH on block liposomes studied by cryogenic transmission electron microscopy[J]. Biochimica et Biophysica Acta-Biomembranes 2009, 1788(9): 1869-1876.
[56] Alfredsson V., Cryo-TEM studies of DNA and DNA-lipid structures[J]. Current Opinion In Colloid & Interface Science 2005, 10(5-6): 269-273.
[57] Tatischeff I.; Larquet E.; Falcon-Perez J. M., et al., Fast characterisation of cell-derived extracellular vesicles by nanoparticles tracking analysis, cryo-electron microscopy, and raman tweezers microspectroscopy[J]. Journal of Extracellular Vesicles 2012, 1(1): 1-11.
[58] Carroni M.; Saibil H. R., Cryo electron microscopy to determine the structure of macromolecular complexes[J]. Methods 2016, 95(1): 78-85.
[59] Bartesaghi A.; Merk A.; Banerjee S., et al., 2.2 A resolution cryo-EM structure of beta-galactosidase in complex with a cell-permeant inhibitor[J]. Science 2015, 348(6239): 1147-1151.
[60] Lengyel J. S.; Milne J. L.; Subramaniam S., Electron tomography in nanoparticle imaging and analysis[J]. Nanomedicine (Lond) 2008, 3(1): 125-131.
[61] Bonnaud C.; Monnier C. A.; Demurtas D., et al., Insertion of nanoparticle clusters into vesicle bilayers[J]. ACS Nano 2014, 8(4): 3451-3460.
[62] Le Bihan O.; Bonnafous P.; Marak L., et al., Cryo-electron tomography of nanoparticle transmigration into liposome[J]. Journal of Structural Biology 2009, 168(3): 419-425.
[63] Binnig G.; Quate C. F.; Gerber C., Atomic force microscope[J]. Physical Review Letters 1986, 56(9): 930-933.
[64] Vahabi S.; Nazemi Salman B.; Javanmard A., Atomic force microscopy application in biological research: A review study[J]. Iranian Journal of Medical Sciences 2013, 38(2): 76-83.
[65] Ruozi B.; Tosi G.; Tonelli M., et al., AFM phase imaging of soft-hydrated samples: A versatile tool to

complete the chemical-physical study of liposomes[J]. Journal of Liposome Research 2009, 19(1): 59-67.
[66] Nakano K.; Tozuka Y.; Yamamoto H., et al., A novel method for measuring rigidity of submicron-size liposomes with atomic force microscopy[J]. International Journal of Pharmaceutics 2008, 355(1-2): 203-209.
[67] Takechi-Haraya Y.; Sakai-Kato K.; Abe Y., et al., Atomic force microscopic analysis of the effect of lipid composition on liposome membrane rigidity[J]. Langmuir 2016, 32(24): 6074-6082.
[68] Anabousi S.; Laue M.; Lehr C. M., et al., Assessing transferrin modification of liposomes by atomic force microscopy and transmission electron microscopy[J]. European Journal of Pharmaceutics and Biopharmaceutics 2005, 60(2): 295-303.
[69] Ramachandran S.; Quist A. P.; Kumar S., et al., Cisplatin nanoliposomes for cancer therapy: AFM and fluorescence imaging of cisplatin encapsulation, stability, cellular uptake, and toxicity[J]. Langmuir 2006, 22(19): 8156-8162.
[70] Kanno T.; Yamada T.; Iwabuki H., et al., Size distribution measurement of vesicles by atomic force microscopy[J]. Analytical Biochemistry 2002, 309(2): 196-199.
[71] Baclayon M.; Roos W. H.; Wuite G. J. L., Sampling protein form and function with the atomic force microscope[J]. Molecular & Cellular Proteomics 2010, 9(8): 1678-1688.
[72] Barlow B. M.; Bertrand M.; Joos B., Relaxation of a simulated lipid bilayer vesicle compressed by an atomic force microscope[J]. Physical Review E 2016, 94(5): 10.
[73] Lichtman J. W.; Conchello J. A., Fluorescence microscopy[J]. Nature Methods 2005, 2(12): 910-919.
[74] Combs C. A., Fluorescence microscopy: A concise guide to current imaging methods[J]. Current Protocols in Neuroscience 2010, 2(1): 1-19.
[75] Leung S. J.; Romanowski M., Light-activated content release from liposomes[J]. Theranostics 2012, 2(10): 1020-1036.
[76] https://en.wikipedia.org/wiki/Fluorescence_microscope.
[77] Kunding A. H.; Mortensen M. W.; Christensen S. M., et al., A fluorescence-based technique to construct size distributions from single-object measurements: Application to the extrusion of lipid vesicles[J]. Biophysical Journal 2008, 95(3): 1176-1188.
[78] Lohse B.; Bolinger P. Y.; Stamou D., Encapsulation efficiency measured on single small unilamellar vesicles[J]. Journal of the American Chemical Society 2008, 130(44): 14372-14373.
[79] Larsen J.; Hatzakis N. S.; Stamou D., Observation of inhomogeneity in the lipid composition of individual nanoscale liposomes[J]. Journal of the American Chemical Society 2011, 133(28): 10685-10687.
[80] Elizondo E.; Larsen J.; Hatzakis N. S., et al., Influence of the preparation route on the supramolecular organization of lipids in a vesicular system[J]. Journal of the American Chemical Society 2012, 134(4): 1918-1921.
[81] Heider E. C.; Barhoum M.; Edwards K., et al., Structural characterization of individual vesicles using fluorescence microscopy[J]. Analytical Chemistry 2011, 83(12): 4909-4915.
[82] Heider E. C.; Peterson E. M.; Barhoum M., et al., Quantitative fluorescence microscopy to determine molecular occupancy of phospholipid vesicles[J]. Analytical Chemistry 2011, 83(13): 5128-5136.
[83] Le Meins J. F.; Schatz C.; Lecommandoux S., et al., Hybrid polymer/lipid vesicles: State of the art and future perspectives[J]. Materials Today 2013, 16(10): 397-402.
[84] Gilleron J.; Querbes W.; Zeigerer A., et al., Image-based analysis of lipid nanoparticle-mediated sirna delivery, intracellular trafficking and endosomal escape[J]. Nature Biotechnology 2013, 31(7): 638-646.
[85] Lugli E.; Roederer M.; Cossarizza A., Data analysis in flow cytometry: The future just started[J]. Cytometry Part A 2010, 77A(7): 705-713.
[86] Adan A.; Alizada G.; Kiraz Y., et al., Flow cytometry: Basic principles and applications[J]. Critical Reviews in Biotechnology 2017, 37(2): 163-176.
[87] http://flowcytometry.med.ualberta.ca/.
[88] Vorauer-Uhl K.; Wagner A.; Borth N., et al., Determination of liposome size distribution by flow cytometry[J]. Cytometry 2000, 39(2): 166-171.

[89] Sato K.; Obinata K.; Sugawara T., et al., Quantification of structural properties of cell-sized individual liposomes by flow cytometry[J]. Journal of Bioscience and Bioengineering 2006, 102(3): 171-178.
[90] Sakakura T.; Nishimura K.; Suzuki H., et al., Statistical analysis of discrete encapsulation of nanomaterials in colloidal capsules[J]. Analytical Methods 2012, 4(6): 1648-1655.
[91] Nordlund G.; Lonneborg R.; Brzezinski P., Formation of supported lipid bilayers on silica particles studied using flow cytometry[J]. Langmuir 2009, 25(8): 4601-4606.
[92] Esposito S.; Colicchia S.; de la Torre X., et al., Liposomes as potential masking agents in sport doping. Part 2: Detection of liposome-entrapped haemoglobin by flow cytofluorimetry[J]. Drug Testing and Analysis 2017, 9(2): 208-215.
[93] Bryla A.; Lewandowicz G.; Juzwa W., Encapsulation of elderberry extract into phospholipid nanoparticles[J]. Journal of Food Engineering 2015, 167(1): 189-195.
[94] van der Vlist E. J.; Nolte-'t Hoen E. N.; Stoorvogel W., et al., Fluorescent labeling of nano-sized vesicles released by cells and subsequent quantitative and qualitative analysis by high-resolution flow cytometry[J]. Nature Protocols 2012, 7(7): 1311-1326.
[95] Malloy A.; Carr B., Nanoparticle tracking analysis - the HaloTM system[J]. Particle & Particle Systems Characterization 2006, 23(2): 197-204.
[96] Saveyn H.; De Baets B.; Thas O., et al., Accurate particle size distribution determination by nanoparticle tracking analysis based on 2-D brownian dynamics simulation[J]. Journal of Colloid and Interface Science 2010, 352(2): 593-600.
[97] Filipe V.; Hawe A.; Jiskoot W., Critical evaluation of nanoparticle tracking analysis (NTA) by nanosight for the measurement of nanoparticles and protein aggregates[J]. Pharmaceutical Research 2010, 27(5): 796-810.
[98] van der Pol E.; Coumans F. A. W.; Sturk A., et al., Refractive index determination of nanoparticles in suspension using nanoparticle tracking analysis[J]. Nano Letters 2014, 14(11): 6195-6201.
[99] Reshetov V.; Zorin V.; Siupa A., et al., Interaction of liposomal formulations of meta-tetra(hydroxyphenyl)chlorin (temoporfin) with serum proteins: Protein binding and liposome destruction[J]. Photochemistry and Photobiology 2012, 88(5): 1256-1264.
[100] Fan Y. C.; Sahdev P.; Ochyl L. J., et al., Cationic liposome-hyaluronic acid hybrid nanoparticles for intranasal vaccination with subunit antigens[J]. Journal of Controlled Release 2015, 208(1): 121-129.
[101] Gardiner C.; Shaw M.; Hole P., et al., Measurement of refractive index by nanoparticle tracking analysis reveals heterogeneity in extracellular vesicles[J]. Journal of Extracellular Vesicles 2014, 3(1): 6.
[102] Sato Y. T.; Umezaki K.; Sawada S., et al., Engineering hybrid exosomes by membrane fusion with liposomes[J]. Scientific Reports 2016, 6(1): 21933.
[103] Braeckmans K.; Buyens K.; Bouquet W., et al., Sizing nanomatter in biological fluids by fluorescence single particle tracking[J]. Nano Letters 2010, 10(11): 4435-4442.
[104] Henriquez R. R.; Ito T.; Sun L., et al., The resurgence of coulter counting for analyzing nanoscale objects[J]. Analyst 2004, 129(6): 478-482.
[105] Kozak D.; Anderson W.; Vogel R., et al., Advances in resistive pulse sensors: Devices bridging the void between molecular and microscopic detection[J]. Nano Today 2011, 6(5): 531-545.
[106] Bayley H.; Martin C. R., Resistive-pulse sensing - from microbes to molecules[J]. Chemical Reviews 2000, 100(7): 2575-2594.
[107] Sowerby S. J.; Broom M. F.; Petersen G. B., Dynamically resizable nanometre-scale apertures for molecular sensing[J]. Sensors and Actuators B-Chemical 2007, 123(1): 325-330.
[108] Blundell E. L. C. J.; Mayne L. J.; Billinge E. R., et al., Emergence of tunable resistive pulse sensing as a biosensor[J]. Analytical Methods 2015, 7(17): 7055-7066.
[109] Weatherall E.; Willmott G. R., Applications of tunable resistive pulse sensing[J]. Analyst 2015, 140(10): 3318-3334.
[110] Kozak D.; Broom M.; Vogel R., High resolution particle characterization to expedite development and regulatory acceptance of nanomedicines[J]. Current Drug Delivery 2015, 12(1): 115-120.

[111] Darvish A.; Goyal G.; Aneja R., et al., Nanoparticle mechanics: Deformation detection via nanopore resistive pulse sensing[J]. Nanoscale 2016, 8(30): 14420-14431.
[112] Shimbo K.; Miyaki S.; Ishitobi H., et al., Exosome-formed synthetic microrna-143 is transferred to osteosarcoma cells and inhibits their migration[J]. Biochemical and Biophysical Research Communications 2014, 445(2): 381-387.
[113] Cheng W.; Compton R. G., Investigation of single-drug-encapsulating liposomes using the nano-impact method[J]. Angewandte Chemie-International Edition 2014, 53(50): 13928-13930.
[114] Xiao X. Y.; Bard A. J., Observing single nanoparticle collisions at an ultramicroelectrode by electrocatalytic amplification[J]. Journal of the American Chemical Society 2007, 129(31): 9610-9612.
[115] Cheng W.; Compton R. G., Measuring the content of a single liposome through electrocatalytic nanoimpact "titrations"[J]. Chemelectrochem 2016, 3(12): 2017-2020.
[116] Dunevall J.; Fathali H.; Najafinobar N., et al., Characterizing the catecholamine content of single mammalian vesicles by collision-adsorption events at an electrode[J]. Journal of the American Chemical Society 2015, 137(13): 4344-4346.
[117] Li X. C.; Majdi S.; Dunevall J., et al., Quantitative measurement of transmitters in individual vesicles in the cytoplasm of single cells with nanotip electrodes[J]. Angewandte Chemie-International Edition 2015, 54(41): 11978-11982.
[118] de Souza T. P.; Fahr A.; Luisi P. L., et al., Spontaneous encapsulation and concentration of biological macromolecules in liposomes: An intriguing phenomenon and its relevance in origins of life[J]. Journal of Molecular Evolution 2014, 79(5-6): 179-192.
[119] Luisi P. L.; Allegretti M.; de Souza T. P., et al., Spontaneous protein crowding in liposomes: A new vista for the origin of cellular metabolism[J]. ChemBioChem 2010, 11(14): 1989-1992.
[120] Block S.; Fast B. J.; Lundgren A., et al., Two-dimensional flow nanometry of biological nanoparticles for accurate determination of their size and emission intensity[J]. Nature Communications 2016, 7(1): 12956.
[121] Cressey D., Tiny traits cause big headaches[J]. Nature 2010, 467(7313): 264-265.
[122] Yang L.; Zhu S.; Hang W., et al., Development of an ultrasensitive dual-channel flow cytometer for the individual analysis of nanosized particles and biomolecules[J]. Analytical Chemistry 2009, 81(7): 2555-2563.
[123] Yang L. L.; Zhou Y. X.; Zhu S. B., et al., Detection and quantification of bacterial autofluorescence at the single-cell level by a laboratory-built high-sensitivity flow cytometer[J]. Analytical Chemistry 2012, 84(3): 1526-1532.
[124] Gunasekera T. S.; Attfield P. V.; Veal D. A., A flow cytometry method for rapid detection and enumeration of total bacteria in milk[J]. Applied and Environmental Microbiology 2000, 66(3): 1228-1232.
[125] Yang L. L.; Wu L. N.; Zhu S. B., et al., Rapid, absolute, and simultaneous quantification of specific pathogenic strain and total bacterial cells using an ultrasensitive dual-color flow cytometer[J]. Analytical Chemistry 2010, 82(3): 1109-1116.
[126] Huang T. X.; Zheng Y.; Yan Y., et al., Probing minority population of antibiotic-resistant bacteria[J]. Biosensors & Bioelectronics 2016, 80(1): 323-330.
[127] Yang L.; Huang T.; Zhu S., et al., High-throughput single-cell analysis of low copy number beta-galactosidase by a laboratory-built high-sensitivity flow cytometer[J]. Biosensors & Bioelectronics 2013, 48(1): 49-55.
[128] Wu L.; Wang X.; Zhang J., et al., Flow cytometric single-cell analysis for quantitative in vivo detection of protein-protein interactions via relative reporter protein expression measurement[J]. Analytical Chemistry 2017, 89(5): 2782-2789.
[129] Zhang S.; Zhu S.; Yang L., et al., High-throughput multiparameter analysis of individual mitochondria[J]. Analytical Chemistry 2012, 84(15): 6421-6428.
[130] Zhang X.; Zhang S. Y.; Zhu S. B., et al., Identification of mitochondria-targeting anticancer compounds by an in vitro strategy[J]. Analytical Chemistry 2014, 86(11): 5232-5237.
[131] Chen C. X.; Zhang X.; Zhang S. Y., et al., Quantification of protein copy number in single mitochondria:

The bcl-2 family proteins[J]. Biosensors & Bioelectronics 2015, 74(1): 476-482.
[132] Zhu S. B.; Yang L. L.; Long Y., et al., Size differentiation and absolute quantification of gold nanoparticles via single particle detection with a laboratory-built high-sensitivity flow cytometer[J]. Journal of the American Chemical Society 2010, 132(35): 12176-12178.
[133] Zhu S. B.; Ma L.; Wang S., et al., Light-scattering detection below the level of single fluorescent molecules for high-resolution characterization of functional nanoparticles[J]. ACS Nano 2014, 8(10): 10998-11006.
[134] Ma L.; Zhu S. B.; Tian Y., et al., Label-free analysis of single viruses with a resolution comparable to that of electron microscopy and the throughput of flow cytometry[J]. Angewandte Chemie-International Edition 2016, 55(35): 10239-10243.
第二章
[1] Shi J.; Kantoff P. W.; Wooster R., et al., Cancer nanomedicine: Progress, challenges and opportunities[J]. Nature Reviews Cancer 2017, 17(1): 20-37.
[2] Blanco E.; Shen H.; Ferrari M., Principles of nanoparticle design for overcoming biological barriers to drug delivery[J]. Nature Biotechnology 2015, 33(9): 941-951.
[3] Bazak R.; Houri M.; Achy S. E., et al., Passive targeting of nanoparticles to cancer: A comprehensive review of the literature[J]. Molecular and Clinical Oncology 2014, 2(6): 904-908.
[4] Nakamura Y.; Mochida A.; Choyke P. L., et al., Nanodrug delivery: Is the enhanced permeability and retention effect sufficient for curing cancer?[J]. Bioconjugate Chemistry 2016, 27(10): 2225-2238.
[5] Hupfeld S.; Holsaeter A. M.; Skar M., et al., Liposome size analysis by dynamic/static light scattering upon size exclusion-/field flow-fractionation[J]. Journal of Nanoscience and Nanotechnology 2006, 6(9-10): 3025-3031.
[6] Richter A. G.; Dergunov S. A.; Ganus B., et al., Scattering studies of hydrophobic monomers in liposomal bilayers: An expanding shell model of monomer distribution[J]. Langmuir 2011, 27(7): 3792-3797.
[7] Lengyel J. S.; Milne J. L. S.; Subramaniam S., Electron tomography in nanoparticle imaging and analysis[J]. Nanomedicine 2008, 3(1): 125-131.
[8] Almgren M.; Edwards K.; Karlsson G., Cryo transmission electron microscopy of liposomes and related structures[J]. Colloids and Surfaces A-Physicochemical and Engineering Aspects 2000, 174(1-2): 3-21.
[9] Yang L.; Broom M. F.; Tucker I. G., Characterization of a nanoparticulate drug delivery system using scanning ion occlusion sensing[J]. Pharmaceutical Research 2012, 29(9): 2578-2586.
[10] Saveyn H.; De Baets B.; Thas O., et al., Accurate particle size distribution determination by nanoparticle tracking analysis based on 2-D brownian dynamics simulation[J]. Journal of Colloid and Interface Science 2010, 352(2): 593-600.
[11] Cressey D., Tiny traits cause big headaches[J]. Nature 2010, 467(7313): 264-265.
[12] Adan A.; Alizada G.; Kiraz Y., et al., Flow cytometry: Basic principles and applications[J]. Critical Reviews in Biotechnology 2017, 37(2): 163-176.
[13] He G. S.; Qin H. Y.; Zheng Q., Rayleigh, mie, and tyndall scatterings of polystyrene microspheres in water: Wavelength, size, and angle dependences[J]. Journal of Applied Physics 2009, 105(2): 023110.
[14] Ma L.; Zhu S. B.; Tian Y., et al., Label-free analysis of single viruses with a resolution comparable to that of electron microscopy and the throughput of flow cytometry[J]. Angewandte Chemie-International Edition 2016, 55(35): 10239-10243.
[15] Zhu S. B.; Ma L.; Wang S., et al., Light-scattering detection below the level of single fluorescent molecules for high-resolution characterization of functional nanoparticles[J]. ACS Nano 2014, 8(10): 10998-11006.
[16] Zhu S. B.; Yang L. L.; Long Y., et al., Size differentiation and absolute quantification of gold nanoparticles via single particle detection with a laboratory-built high-sensitivity flow cytometer[J]. Journal of the American Chemical Society 2010, 132(35): 12176-12178.
[17] Yang L.; Zhu S.; Hang W., et al., Development of an ultrasensitive dual-channel flow cytometer for the individual analysis of nanosized particles and biomolecules[J]. Analytical Chemistry 2009, 81(7): 2555-2563.
[18] Barenholz Y., Doxil(R)--the first FDA-approved nano-drug: Lessons learned[J]. Journal of Controlled

Release 2012, 160(2): 117-134.
[19] Xie R.; Hong S. L.; Feng L. S., et al., Cell-selective metabolic glycan labeling based on ligand-targeted liposomes[J]. Journal of the American Chemical Society 2012, 134(24): 9914-9917.
[20] Berger N.; Sachse A.; Bender J., et al., Filter extrusion of liposomes using different devices: Comparison of liposome size, encapsulation efficiency, and process characteristics[J]. International Journal of Pharmaceutics 2001, 223(1-2): 55-68.
[21] Akbarzadeh A.; Rezaei-Sadabady R.; Davaran S., et al., Liposome: Classification, preparation, and applications[J]. Nanoscale Research Letters 2013, 8(1): 102-110.
[22] Stewart J. C. M., Colorimetric determination of phospholipids with ammonium ferrothiocyanate[J]. Analytical Biochemistry 1980, 104(1): 10-14.
[23] Castile J. D.; Taylor K. M. G., Factors affecting the size distribution of liposomes produced by freeze-thaw extrusion[J]. International Journal of Pharmaceutics 1999, 188(1): 87-95.
[24] Barba A. A.; Bochicchio S.; Lamberti G., et al., Ultrasonic energy in liposome production: Process modelling and size calculation[J]. Soft Matter 2014, 10(15): 2574-2581.
[25] Morton L. A.; Saludes J. P.; Yin H., Constant pressure-controlled extrusion method for the preparation of nano-sized lipid vesicles[J]. Jove-Journal of Visualized Experiments 2012, 64(1): e4151.
[26] Edwards K. A.; Baeumner A. J., Analysis of liposomes[J]. Talanta 2006, 68(5): 1432-1441.
[27] Gubernator J., Active methods of drug loading into liposomes: Recent strategies for stable drug entrapment and increased in vivo activity[J]. Expert Opinion on Drug Delivery 2011, 8(5): 565-580.
[28] Kibria G.; Hatakeyama H.; Ohga N., et al., The effect of liposomal size on the targeted delivery of doxorubicin to integrin alpha v beta 3-expressing tumor endothelial cells[J]. Biomaterials 2013, 34(22): 5617-5627.
[29] Maruyama K., Intracellular targeting delivery of liposomal drugs to solid tumors based on EPR effects[J]. Advanced Drug Delivery Reviews 2011, 63(3): 161-169.
[30] Antonietti M.; Forster S., Vesicles and liposomes: A self-assembly principle beyond lipids[J]. Advanced Materials 2003, 15(16): 1323-1333.
[31] van der Pol E.; Coumans F. A. W.; Sturk A., et al., Refractive index determination of nanoparticles in suspension using nanoparticle tracking analysis[J]. Nano Letters 2014, 14(11): 6195-6201.
[32] Busse S.; Scheumann V.; Menges B., et al., Sensitivity studies for specific binding reactions using the biotin/streptavidin system by evanescent optical methods[J]. Biosensors & Bioelectronics 2002, 17(8): 704-710.
[33] Hupfeld S.; Holsaeter A. M.; Skar M., et al., Liposome size analysis by dynamic/static light scattering upon size exclusion-/field flow-fractionation[J]. Journal of Nanoscience and Nanotechnology 2006, 6(9-10): 3025-3031.
[34] Yoshikawa W.; Akutsu H.; Kyogoku Y., Light-scattering properties of osmotically active liposomes[J]. Biochimica et Biophysica Acta 1983, 735(3): 397-406.
[35] Matsuzaki K.; Murase O.; Sugishita K., et al., Optical characterization of liposomes by right angle light scattering and turbidity measurement[J]. Biochimica et Biophysica Acta-Biomembranes 2000, 1467(1): 219-226.
[36] Chong C. S.; Colbow K., Light scattering and turbidity measurements on lipid vesicles[J]. Biochimica et Biophysica Acta 1976, 436(2): 260-282.
[37] Zasadzinski J. A.; Wong B.; Forbes N., et al., Novel methods of enhanced retention in and rapid, targeted release from liposomes[J]. Current Opinion in Colloid & Interface Science 2011, 16(3): 203-214.
[38] Souza T. G. F.; Ciminelli V. S. T.; Mohallem N. D. S., A comparison of TEM and DLS methods to characterize size distribution of ceramic nanoparticles [J]. Journal of Physics: Conference Series 2016, 733(1): 1-5.
[39] Wibroe P. P.; Ahmadvand D.; Oghabian M. A., et al., An integrated assessment of morphology, size, and complement activation of the PEGylated liposomal doxorubicin products Doxil (R), Caelyx (R), DOXOrubicin, and SinaDoxosome[J]. Journal of Controlled Release 2016, 221(1): 1-8.
[40] O'Shaughnessy J., Liposomal anthracyclines for breast cancer: Overview[J]. Oncologist 2003, 8(12): 32-40.

[41] Allen T. M.; Martin F. J., Advantages of liposomal delivery systems for anthracyclines[J]. Seminars in Oncology 2004, 31(13): 5-15.

第三章

[1] Shi J.; Kantoff P. W.; Wooster R., et al., Cancer nanomedicine: Progress, challenges and opportunities[J]. Nature Reviews Cancer 2017, 17(1): 20-37.
[2] Caster J. M.; Patel A. N.; Zhang T., et al., Investigational nanomedicines in 2016: A review of nanotherapeutics currently undergoing clinical trials[J]. Wiley Interdisciplinary Reviews. Nanomedicine and Nanobiotechnology 2017, 9(1): 1-18.
[3] Xu X.; Ho W.; Zhang X., et al., Cancer nanomedicine: From targeted delivery to combination therapy[J]. Trends Mol Med 2015, 21(4): 223-232.
[4] Rizzo L. Y.; Theek B.; Storm G., et al., Recent progress in nanomedicine: Therapeutic, diagnostic and theranostic applications[J]. Current Opinion in Biotechnology 2013, 24(6): 1159-1166.
[5] Adan A.; Alizada G.; Kiraz Y., et al., Flow cytometry: Basic principles and applications[J]. Critical Reviews in Biotechnology 2017, 37(2): 163-176.
[6] Mohan P.; Rapoport N., Doxorubicin as a molecular nanotheranostic agent: Effect of doxorubicin encapsulation in micelles or nanoemulsions on the ultrasound-mediated intracellular delivery and nuclear trafficking[J]. Molecular Pharmaceutics 2010, 7(6): 1959-1973.
[7] Ishida T.; Kirchmeier M. J.; Moase E. H., et al., Targeted delivery and triggered release of liposomal doxorubicin enhances cytotoxicity against human B lymphoma cells[J]. Biochimica et Biophysica Acta-Biomembranes 2001, 1515(2): 144-158.
[8] Zhdanov V. P.; Cho N. J., Kinetics of the formation of a protein corona around nanoparticles[J]. Mathematical Biosciences 2016, 282(1): 82-90.
[9] Del Pino P.; Pelaz B.; Zhang Q., et al., Protein corona formation around nanoparticles - from the past to the future[J]. Materials Horizons 2014, 1(3): 301-313.
[10] Ritz S.; Schottler S.; Kotman N., et al., Protein corona of nanoparticles: Distinct proteins regulate the cellular uptake[J]. Biomacromolecules 2015, 16(4): 1311-1321.
[11] Ali N.; Mattsson K.; Rissler J., et al., Analysis of nanoparticle-protein coronas formed in vitro between nanosized welding particles and nasal lavage proteins[J]. Nanotoxicology 2016, 10(2): 226-234.
[12] Kuyper C. L.; Budzinski K. L.; Lorenz R. M., et al., Real-time sizing of nanoparticles in microfluidic channels using confocal correlation spectroscopy[J]. Journal of the American Chemical Society 2006, 128(3): 730-731.
[13] Yu M. X.; Wu L. N.; Huang T. X., et al., Rapid detection and enumeration of total bacteria in drinking water and tea beverages using a laboratory-built high-sensitivity flow cytometer[J]. Analytical Methods 2015, 7(7): 3072-3079.
[14] Zhu S. B.; Yang L. L.; Long Y., et al., Size differentiation and absolute quantification of gold nanoparticles via single particle detection with a laboratory-built high-sensitivity flow cytometer[J]. Journal of the American Chemical Society 2010, 132(35): 12176-12178.
[15] Tazina E. V.; Kostin K. V.; Oborotova N. A., Specific features of drug encapsulation in liposomes (a review)[J]. Pharmaceutical Chemistry Journal 2011, 45(8): 481-490.
[16] Lengyel J. S.; Milne J. L. S.; Subramaniam S., Electron tomography in nanoparticle imaging and analysis[J]. Nanomedicine 2008, 3(1): 125-131.
[17] Whitehead K. A.; Langer R.; Anderson D. G., Knocking down barriers: Advances in sirna delivery[J]. Nature Reviews Drug Discovery 2009, 8(2): 129-138.
[18] Zatsepin T. S.; Kotelevtsev Y. V.; Koteliansky V., Lipid nanoparticles for targeted sirna delivery - going from bench to bedside[J]. International Journal of Nanomedicine 2016, 11(1): 3077-3086.
[19] Kanasty R.; Dorkin J. R.; Vegas A., et al., Delivery materials for sirna therapeutics[J]. Nature Materials 2013, 12(11): 967-977.
[20] Naldini L., Gene therapy returns to centre stage[J]. Nature 2015, 526(7573): 351-360.

[21] Wilson R. C.; Doudna J. A., Molecular mechanisms of RNA interference[J]. Annual Review of Biophysics 2013, 42(1): 217-239.
[22] Resnier P.; Montier T.; Mathieu V., et al., A review of the current status of siRNA nanomedicines in the treatment of cancer[J]. Biomaterials 2013, 34(27): 6429-6443.
[23] http://www.alnylam.com/.
[24] Leung A. K. K.; Hafez I. M.; Baoukina S., et al., Lipid nanoparticles containing siRNA synthesized by microfluidic mixing exhibit an electron-dense nanostructured core[J]. Journal of Physical Chemistry C 2012, 116(34): 18440-18450.
第四章
[1] Huynh E.; Zheng G., Cancer nanomedicine: Addressing the dark side of the enhanced permeability and retention effect[J]. Nanomedicine 2015, 10(13): 1993-1995.
[2] Nakamura Y.; Mochida A.; Choyke P. L., et al., Nanodrug delivery: Is the enhanced permeability and retention effect sufficient for curing cancer?[J]. Bioconjugate Chemistry 2016, 27(10): 2225-2238.
[3] Bae Y. H.; Park K., Targeted drug delivery to tumors: Myths, reality and possibility[J]. Journal of Controlled Release 2011, 153(3): 198-205.
[4] Varner C. T.; Rosen T.; Martin J. T., et al., Recent advances in engineering polyvalent biological interactions[J]. Biomacromolecules 2015, 16(1): 43-55.
[5] Srinivasarao M.; Galliford C. V.; Low P. S., Principles in the design of ligand-targeted cancer therapeutics and imaging agents[J]. Nature Reviews Drug Discovery 2015, 14(3): 203-219.
[6] Schrama D.; Reisfeld R. A.; Becker J. C., Antibody targeted drugs as cancer therapeutics[J]. Nature Reviews Drug Discovery 2006, 5(2): 147-159.
[7] Vhora I.; Patil S.; Bhatt P., et al., Receptor-targeted drug delivery: Current perspective and challenges[J]. Therapeutic Delivery 2014, 5(9): 1007-1024.
[8] Irvine D. J., Drug delivery: One nanoparticle, one kill[J]. Nature Materials 2011, 10(5): 342-343.
[9] Wang B.; Galliford C. V.; Low P. S., Guiding principles in the design of ligand-targeted nanomedicines[J]. Nanomedicine (Lond) 2014, 9(2): 313-330.
[10] Zhang Y. H.; Thomas T. P.; Lee K. H., et al., Polyvalent saccharide-functionalized generation 3 poly(amidoamine) dendrimer-methotrexate conjugate as a potential anticancer agent[J]. Bioorganic & Medicinal Chemistry 2011, 19(8): 2557-2564.
[11] Li M. H.; Choi S. K.; Thomas T. P., et al., Dendrimer-based multivalent methotrexates as dual acting nanoconjugates for cancer cell targeting[J]. European Journal of Medicinal Chemistry 2012, 47(1): 560-572.
[12] Zong H.; Thomas T. P.; Lee K. H., et al., Bifunctional pamam dendrimer conjugates of folic acid and methotrexate with defined ratio[J]. Biomacromolecules 2012, 13(4): 982-991.
[13] Smith A. M.; Johnston K. A.; Crawford S. E., et al., Ligand density quantification on colloidal inorganic nanoparticles[J]. Analyst 2017, 142(1): 11-29.
[14] Lengyel J. S.; Milne J. L. S.; Subramaniam S., Electron tomography in nanoparticle imaging and analysis[J]. Nanomedicine 2008, 3(1): 125-131.
[15] Ruozi B.; Tosi G.; Tonelli M., et al., AFM phase imaging of soft-hydrated samples: A versatile tool to complete the chemical-physical study of liposomes[J]. Journal of Liposome Research 2009, 19(1): 59-67.
[16] Kunding A. H.; Mortensen M. W.; Christensen S. M., et al., A fluorescence-based technique to construct size distributions from single-object measurements: Application to the extrusion of lipid vesicles[J]. Biophysical Journal 2008, 95(3): 1176-1188.
[17] Sakakura T.; Nishimura K.; Suzuki H., et al., Statistical analysis of discrete encapsulation of nanomaterials in colloidal capsules[J]. Analytical Methods 2012, 4(6): 1648-1655.
[18] Reshetov V.; Zorin V.; Siupa A., et al., Interaction of liposomal formulations of meta-tetra(hydroxyphenyl)chlorin (temoporfin) with serum proteins: Protein binding and liposome destruction[J]. Photochemistry and Photobiology 2012, 88(5): 1256-1264.
[19] Darvish A.; Goyal G.; Aneja R., et al., Nanoparticle mechanics: Deformation detection via nanopore

resistive pulse sensing[J]. Nanoscale 2016, 8(30): 14420-14431.
[20] Saul J. M.; Annapragada A.; Natarajan J. V., et al., Controlled targeting of liposomal doxorubicin via the folate receptor in vitro[J]. Journal of Controlled Release 2003, 92(1-2): 49-67.
[21] Xu S. L.; Liu Y.; Tai H. C., et al., Synthesis of transferrin (tf) conjugated liposomes via staudinger ligation[J]. International Journal of Pharmaceutics 2011, 404(1-2): 205-210.
[22] Leamon C. P.; Reddy J. A.; Dorton R., et al., Impact of high and low folate diets on tissue folate receptor levels and antitumor responses toward folate-drug conjugates[J]. Journal of Pharmacology and Experimental Therapeutics 2008, 327(3): 918-925.
[23] Bailey L. B.; Gregory J. F., Folate metabolism and requirements[J]. Journal of Nutrition 1999, 129(4): 779-782.
[24] Stover P. J., Physiology of folate and vitamin B12 in health and disease[J]. Nutrition Reviews 2004, 62(6): 3-12.
[25] Elnakat H.; Ratnam M., Distribution, functionality and gene regulation of folate receptor isoforms: Implications in targeted therapy[J]. Advanced Drug Delivery Reviews 2004, 56(8): 1067-1084.
[26] Zhao X.; Li H.; Lee R. J., Targeted drug delivery via folate receptors[J]. Expert Opinion on Drug Delivery 2008, 5(3): 309-319.
[27] Chen C.; Ke J.; Zhou X. E., et al., Structural basis for molecular recognition of folic acid by folate receptors[J]. Nature 2013, 500(7463): 486-489.
[28] Perfetto S. P.; Chattopadhyay P. K.; Roederer M., Seventeen-colour flow cytometry: Unravelling the immune system[J]. Nature Reviews Immunology 2004, 4(8): 648-655.
[29] Hackstadt T., Steric hindrance of antibody binding to surface proteins of coxiella burnetti by phase I lipopolysaccharide[J]. Infection and Immunity 1988, 56(4): 802-807.
[30] Meyboom A.; Maretzki D.; Stevens P. A., et al., Reversible calcium-dependent interaction of liposomes with pulmonary surfactant protein a. Analysis by resonant mirror technique and near-infrared light scattering[J]. Journal of Biological Chemistry 1997, 272(23): 14600-14605.
[31] Chaudhury A.; Das S., Folate receptor targeted liposomes encapsulating anti-cancer drugs[J]. Current Pharmaceutical Biotechnology 2015, 16(4): 333-343.
[32] Ulrich C. M., Folate and cancer prevention: A closer look at a complex picture[J]. American Journal of Clinical Nutrition 2007, 86(2): 271-273.
[33] Marchetti C.; Palaia I.; Giorgini M., et al., Targeted drug delivery via folate receptors in recurrent ovarian cancer: A review[J]. Oncotargets and Therapy 2014, 7(1): 1223-1236.
[34] Albanese A.; Tang P. S.; Chan W. C. W., The effect of nanoparticle size, shape, and surface chemistry on biological systems[J]. Annual Review of Biomedical Engineering 2012, 14(1): 1-16.
[35] Sykes E. A.; Chen J.; Zheng G., et al., Investigating the impact of nanoparticle size on active and passive tumor targeting efficiency[J]. ACS Nano 2014, 8(6): 5696-5706.
[36] Jiang W.; Kim B. Y. S.; Rutka J. T., et al., Nanoparticle-mediated cellular response is size-dependent[J]. Nature Nanotechnology 2008, 3(3): 145-150.
[37] Nogueira E.; Gomes A. C.; Preto A., et al., Design of liposomal formulations for cell targeting[J]. Colloids and Surfaces B-Biointerfaces 2015, 136(1): 514-526.
[38] Maherani B.; Arab-Tehrany E.; Mozafari M. R., et al., Liposomes: A review of manufacturing techniques and targeting strategies[J]. Current Nanoscience 2011, 7(3): 436-452.
[39] Jokerst J. V.; Lobovkina T.; Zare R. N., et al., Nanoparticle pegylation for imaging and therapy[J]. Nanomedicine 2011, 6(4): 715-728.
[40] Parveen S.; Sahoo S. K., Nanomedicine - clinical applications of polyethylene glycol conjugated proteins and drugs[J]. Clinical Pharmacokinetics 2006, 45(10): 965-988.
[41] Steichen S. D.; Caldorera-Moore M.; Peppas N. A., A review of current nanoparticle and targeting moieties for the delivery of cancer therapeutics[J]. European Journal of Pharmaceutical Sciences 2013, 48(3): 416-427.
[42] Hak S.; Helgesen E.; Hektoen H. H., et al., The effect of nanoparticle polyethylene glycol surface density on

ligand-directed tumor targeting studied in vivo by dual modality imaging[J]. ACS Nano 2012, 6(6): 5648-5658.
[43] Noble G. T.; Stefanick J. F.; Ashley J. D., et al., Ligand-targeted liposome design: Challenges and fundamental considerations[J]. Trends in Biotechnology 2014, 32(1): 32-45.
[44] Kawano K.; Maitani Y., Effects of polyethylene glycol spacer length and ligand density on folate receptor targeting of liposomal doxorubicin in vitro[J]. Journal of Drug Delivery 2011, 2011(2011): 160967.
[45] Kim D.; Friedman A. D.; Liu R., Tetraspecific ligand for tumor-targeted delivery of nanomaterials[J]. Biomaterials 2014, 35(23): 6026-6036.
[46] Li X.; Zhao Q.; Qiu L., Smart ligand: Aptamer-mediated targeted delivery of chemotherapeutic drugs and siRNA for cancer therapy[J]. Journal of Controlled Release 2013, 171(2): 152-162.
[47] Singh M., Transferrin as a targeting ligand for liposomes and anticancer drugs[J]. Current Pharmaceutical Design 1999, 5(6): 443-451.
[48] Jin S. E.; Jin H. E.; Hong S. S., Targeted delivery system of nanobiomaterials in anticancer therapy: From cells to clinics[J]. Biomed Research International 2014, 2014(2014): 814208.
[49] Gkouvatsos K.; Papanikolaou G.; Pantopoulos K., Regulation of iron transport and the role of transferrin[J]. Biochimica et Biophysica Acta General Subjects 2012, 1820(3): 188-202.
[50] Tortorella S.; Karagiannis T. C., Transferrin receptor-mediated endocytosis: A useful target for cancer therapy[J]. Journal of Membrane Biology 2014, 247(4): 291-307.
[51] Daniels T. R.; Bernabeu E.; Rodriguez J. A., et al., The transferrin receptor and the targeted delivery of therapeutic agents against cancer[J]. Biochimica et Biophysica Acta General Subjects 2012, 1820(3): 291-317.
[52] Rana S.; Yeh Y. C.; Rotello V. M., Engineering the nanoparticle-protein interface: Applications and possibilities[J]. Current Opinion in Chemical Biology 2010, 14(6): 828-834.
[53] Marques-Gallego P.; de Kroon A. I. P. M., Ligation strategies for targeting liposomal nanocarriers[J]. Biomed Research International 2014, 2014(2014): 129458
[54] Riener C. K.; Kada G.; Gruber H. J., Quick measurement of protein sulfhydryls with Ellman's reagent and with 4,4'-dithiodipyridine[J]. Analytical and Bioanalytical Chemistry 2002, 373(4-5): 266-276.
[55] Brown R. E.; Jarvis K. L.; Hyland K. J., Protein measurement using bicinchoninic acid - elimination of interfering substances[J]. Analytical Biochemistry 1989, 180(1): 136-139.
[56] Sassoon I.; Blanc V., Antibody-drug conjugate (ADC) clinical pipeline: A review[J]. Methods in Molecular Biology 2013, 1045(1): 1-27.
[57] Diamantis N.; Banerji U., Antibody-drug conjugates--an emerging class of cancer treatment[J]. British Journal of Cancer 2016, 114(4): 362-367.
[58] Gao J.; Feng S. S.; Guo Y., Antibody engineering promotes nanomedicine for cancer treatment[J]. Nanomedicine (Lond) 2010, 5(8): 1141-1145.
[59] Krishnamurti U.; Silverman J. F., HER2 in breast cancer: A review and update[J]. Advances in Anatomic Pathology 2014, 21(2): 100-107.
[60] Tai W. Y.; Mahato R.; Cheng K., The role of HER2 in cancer therapy and targeted drug delivery[J]. Journal of Controlled Release 2010, 146(3): 264-275.

第五章

[1] Dan N., Effect of liposome charge and PEG polymer layer thickness on cell-liposome electrostatic interactions[J]. Biochimica et Biophysica Acta 2002, 1564(2): 343-348.
[2] Baptista A. L.; Coutinho P. J.; Real Oliveira M. E., et al., Effect of pH on the control release of microencapsulated dye in lecithin liposomes[J]. Journal of Liposome Research 2003, 13(2): 123-130.
[3] Chandaroy P.; Sen A.; Hui S. W., Temperature-controlled content release from liposomes encapsulating pluronic F127[J]. Journal of Controlled Release 2001, 76(1-2): 27-37.
[4] Corbo C.; Molinaro R.; Taraballi F., et al., Effects of the protein corona on liposome-liposome and liposome-cell interactions[J]. International Journal of Nanomedicine 2016, 11(1): 3049-3063.
[5] Rocker C.; Potzl M.; Zhang F., et al., A quantitative fluorescence study of protein monolayer formation on colloidal nanoparticles[J]. Nature Nanotechnology 2009, 4(9): 577-580.

[6] Luisi P. L.; Stano P.; de Souza T., Spontaneous overcrowding in liposomes as possible origin of metabolism[J]. Origins of Life and Evolution of Biospheres 2014, 44(4): 313-317.
[7] de Souza T. P.; Fahr A.; Luisi P. L., et al., Spontaneous encapsulation and concentration of biological macromolecules in liposomes: An intriguing phenomenon and its relevance in origins of life[J]. Journal of Molecular Evolution 2014, 79(5-6): 179-192.
[8] Stano P., Minimal cells: Relevance and interplay of physical and biochemical factors[J]. Biotechnology Journal 2011, 6(7): 850-859.
[9] Stano P.; Luisi P. L., Semi-synthetic minimal cells: Origin and recent developments[J]. Current Opinion in Biotechnology 2013, 24(4): 633-638.
[10] Kuruma Y.; Ueda T., The PURE system for the cell-free synthesis of membrane proteins[J]. Nature Protocols 2015, 10(9): 1328-1344.
[11] Mathiasen S.; Christensen S. M.; Fung J. J., et al., Nanoscale high-content analysis using compositional heterogeneities of single proteoliposomes[J]. Nature Methods 2014, 11(9): 931-934.