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基于多肽和蛋白质分子的纳米生物界面效应及其应用研究

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摘要 通过对纳米结构进行表面生物和化学修饰, 能够赋予其崭新的界面性质. 本文概述了纳米颗粒表面共价修饰和非共价修饰多肽和蛋白质的常用方法, 对比了两种修饰方法的优缺点以及构筑纳米生物结构存在的问题; 并介绍了多肽和蛋白质界面修饰在改善纳米颗粒生物稳定性、生物分布和靶向性方面的研究工作; 在此基础上介绍了纳米生物结构基于抗原-抗体特异性识别在生物检测领域的应用; 此外, 简单介绍了纳米生物结构在应用过程中所面临的挑战. 希望本综述能够有助于科技工作者了解纳米生物结构的构筑方法及其应用方面的进展和挑战, 为多肽和蛋白质修饰纳米结构的设计合成提供一些启发和思路.

关键词 纳米颗粒, 多肽, 蛋白质, 界面修饰, 生物分布, 靶向性, 生物检测

纳米结构由于其独特的物理和化学性质以及较为完备的合成技术在生物医药领域受到越来越多的关注, 目前已逐步应用于药物递送、生物成像和生物检测等临床研究中. 纳米结构在生物系统中的重要性质, 如纳米生物界面的结构和性质, 也成为广泛关注的研究热点, 并对其在生物医药领域的应用具有重要意义. 纳米生物界面的研究对于认识相关纳米结构与生物系统的相互作用具有基础性的意义, 为发展纳米生物技术提供了重要基础. 这方面的主要科学挑战在于从原子和分子水平上认识液固界面结构、纳米结构与生物分子的相互作用(包括疏水作用、氢键、静电作用等)以及纳米生物界面化学组分、分布特征和调控机制. 这些研究对于深入认识生物系统中纳米结构及界面的转变与演化规律和纳米结构的生物效应与分子机制具有关键意义, 也有助于发展疾病诊断和治疗的新型纳米技术.

应用于生物系统中的纳米结构包括多种类型. 金纳米颗粒易于制备, 形状和尺寸可控, 有很好的生物相容性, 其表面易于修饰, 可以通过Au–S键与各种药物分子和生物分子结合, 是一种高效的载体[1]. 金纳米粒子能够吸收大量的X射线, 再加上其独特的光学性质: 局域表面等离激元共振(LSPR)效应, 使其在肿瘤治疗、光学传感、生物成像等领域有很大的应用前景[1,2]. 量子点也是一类具有独特光学性质的新型半导体荧光纳米材料. 与传统的荧光染料相比, 其具有激发光谱宽、发射光谱窄、量子产率高和光稳定性好等优点, 并且其发射光谱可以通过改变尺寸调控至近红外区, 有助于体内实验研究[3]. 目前量子点已被广泛用于生物标记、生物传感和生物成像中[4,5]. 介孔氧化硅纳米粒子也是一类重要的纳米材料, 它具有很大的孔容量和比表面积, 并且有均一的孔道分布和可控的孔径大小, 是一种高效的载体; 其

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表面有大量的硅羟基, 易于表面修饰, 通过表面修饰能够调控药物递送的靶向性和可控释放, 此类材料已在药物递送和生物成像等领域有广泛应用[6~8]. 此外, 磁性纳米颗粒由于能够在外加磁场的作用下实现定向移动, 方便定位和分离, 目前也已应用于磁共振成像(MRI)的造影剂[9,10]、药物载体[9,11]、磁热疗[11,12]以及细胞、DNA和蛋白质的提纯、富集和分离[9]等方面. 除了上述介绍的几种纳米材料以外, 上转换发光纳米材料[13,14]、脂质体[15,16]、聚合物纳米颗粒[17]等纳米结构也广泛应用于生物医药领域.

表面修饰能够赋予纳米结构崭新的界面性质, 在纳米结构的表面生物和化学修饰研究中, 利用多肽或蛋白质构筑纳米生物结构是十分活跃的热点方向之一. 研究表明多肽和蛋白质能够提高纳米粒子的分散性和生物稳定性; 跨膜肽的存在能够有效改善纳米结构的生物分布; 基于抗原-抗体等生物分子间的特异性识别能够提高纳米结构靶向性, 并且基于多肽和蛋白质间的特异性识别拓展了纳米结构在生物检测领域的应用. 本文概述了多肽和蛋白质与纳米颗粒构筑纳米生物结构的方法, 介绍了基于多肽和蛋白质表面修饰在改善纳米颗粒稳定性、生物分布、靶向性方面的研究工作, 在此基础上介绍了纳米生物结构基于抗原-抗体特异性识别在生物检测领域的应用.

1 多肽/蛋白质与纳米颗粒复合结构的构筑

1.1 多肽/蛋白质与纳米颗粒的共价连接

多肽和蛋白质通过共价连接的方式能够与不同类型的纳米颗粒形成复合结构. 金纳米颗粒可以通过Au–S键与多肽或蛋白质共价连接. 如果多肽、蛋白质的氨基酸序列中有半胱氨酸, 则可以直接与金纳米粒子共价结合[18], 但这样的结合很可能会影响多肽和蛋白质的生物活性[19], 所以通常会在纳米粒子和活性生物分子间加一个带巯基的连接段, 常用的巯基化合物有半胱氨酸、巯基丙酸、谷胱甘肽、胱胺等[20]. 单个巯基连接存在不稳定性, 当溶液中有较高浓度的其他巯基化合物时, 纳米粒子上连接的生物分子会被溶液中的巯基化合物取代, 这种情况可以通过增加单个连接段上的巯基个数来增加多肽和蛋白质在纳米粒子上的连接稳定性[21,22]. 同理, 量子点也可以通过巯基与Zn, Cd等金属间的强相互作用, 利用巯基乙酸、巯基丙酸等巯基化合物在量子点表面引入官能团, 以便与多肽、蛋白质共价结合[23]. 介孔氧化硅纳米颗粒可通过表面大量的硅羟基与带官能团的硅烷偶联剂反应在纳米颗粒表面引入具有反应活性的官能团[24]. 脂质体则主要通过末端带有活性官能团的两亲性分子与多肽和蛋白质共价偶联, 两亲性分子的长链烷烃通过疏水作用插入脂质体膜内, 而亲水部分的官能团暴露在脂质体表面以便与多肽和蛋白质连接[25~27]. 除了上述连接方法, 纳米颗粒还可以通过在表面包裹功能化的聚乙二醇(PEG)、聚乙烯醇(PVA)等高分子聚合物[28~30]、二氧化硅[31~34]等结构与多肽和蛋白质共价结合. 共价连接一般是通过多肽和蛋白质上的氨基、羧基或者巯基与纳米粒子表面的官能团反应. 最常用的方法是利用1-(3-二甲氨基丙基)-3-乙基碳二亚胺盐酸盐(EDC)与*N*-羟基琥珀酰亚胺(NHS)连用活化羧基后与氨基反应, 但是该反应受环境pH影响较大, 活化后的中间体易水解, 产率相对较低, 还会得到生物分子相互交联的副产物, 如果多肽和蛋白质上存在多个上述基团也会导致其与纳米结构连接方式的多样性, 进而影响其生物活性[35~37]. 有研究者利用点击反应(“click” reactions)使纳米结构与多肽和蛋白质共价连接, 并且通过正交点击反应(orthogonal “click” reactions)构筑多功能的纳米生物结构[38,39]. 这种共价连接方式需在多肽和蛋白质上引入官能团, 比如叠氮基、烯基和巯基等. 除巯基外, 其他引入的官能团都是多肽和蛋白质中自然不存在的, 因此选择性高, 生物分子在纳米界面的取向更可控, 反应得到的副产物更少, 且此方法反应效率高、稳定性好, 但是反应所需时间长[40~42]. 目前已有多种类型的点击反应成功用于纳米结构与多肽和蛋白质的共价连接[43], 在实验中应根据实际情况谨慎选择, 比如铜催化的叠氮炔环加成反应(CuAAC)中用到的铜催化剂有细胞毒性, 能够使蛋白质变性, 并且会降低量子点的量子产率[43,44]. 总之, 发展纳米结构与生物分子的共价连接方法除了要考虑反应时间、反应选择性和产率等问题, 还应考虑反应条件及反应中所用试剂对生物分子和纳米结构性质的影响.

1.2 多肽/蛋白质与纳米颗粒的非共价结合

非共价结合操作简单, 最直接的方式就是让多肽或蛋白质通过物理吸附结合在纳米粒子表面, 这

种方式的结合位点不可控，往往会使生物分子的结构发生改变，进而导致其活性降低或丧失[45]，还可能会因为上述的竞争性取代而发生脱附. 静电、疏水和π-π堆积等作用力以及纳米结构的尺寸在多肽和蛋白质与纳米界面的相互作用中发挥了重要作用[46~50]，所以可以通过调控纳米粒子表面的物理化学性质和其尺寸来调控多肽和蛋白质与表面的相互作用，以维持生物分子的活性和实现吸附构象的专一性. 例如，在硒化镉(CdSe)纳米粒子表面修饰一层末端羧基化的聚乙二醇低聚物(OEG)，糜蛋白质酶通过静电相互作用与纳米粒子紧密结合，在高离子强度的溶液中脱附后仍能保持其活性[51]. 在金纳米粒子表面修饰末端带有氨基酸的聚乙二醇低聚物，可以通过氨基酸侧链的亲疏水性来控制细胞色素*c*(Cytochrome *c*)与纳米粒子的结合区域[52]. 但是由于对多肽和蛋白质在纳米界面的吸附行为缺乏深入认识，所以目前对于其在表面的取向调控仍然具有很大难度. 除了上述的物理吸附外，非共价结合中还存在一些高特异性和高稳定性的连接方式，比如生物素(Biotin)和链霉亲和素(Streptavidin)的强相互作用、蛋白质A(Protein A)与免疫球蛋白的特异性识别. Biotin与Streptavidin的结合具有高度特异性和稳定性，是已知强度最高的非共价作用，纳米粒子与多肽和蛋白质通过Streptavidin-Biotin结合是目前使用最多的非共价结合方式[36]. 蛋白质A可以与抗体的Fc段特异性结合，这种非共价结合方式能够维持抗体的生物活性[36]. 此外，对于一些含金属的纳米粒子，比如金、氧化铁、量子点等纳米粒子，多肽和蛋白质也可以通过配位作用与纳米粒子非共价结合. 常用的方法是用组氨酸标记多肽和蛋白质，通过组氨酸上的咪唑基与金属的配位作用连接在纳米粒子上[22,53,54].

纳米生物结构的构筑即构建一个新的纳米生物界面，进而赋予纳米结构新的生物学功能. 多肽和蛋白质与纳米结构结合后必须保证完整的活性位点和正确的取向才能挥发其功能. 多肽和蛋白质可以通过共价键与纳米粒子连接，也可以通过非共价相互作用结合在纳米粒子表面，这两种连接方法有各自的优缺点，目前最常用的还是前者. 相比非共价结合，共价连接更稳定，当纳米粒子要用于血液、脑脊液等复杂环境中时，这一优势更突出，因为通过非共价相互作用吸附在纳米粒子上的多肽和蛋白质很可能会被复杂环境中各种各样的蛋白质所取代[55]. 共价连接的位点也更可控，当连接的多肽和蛋白质是靶向分子时，比如抗体，必须要使其活性部位不被破坏和掩盖，连接位点可控就显得尤为重要. 但是仍然存在一些特例[6,35,56]，比如最新的研究表明，通过物理吸附结合在聚苯乙烯纳米粒子表面的抗体比通过共价键连接在粒子表面的抗体活性更高，并且即使在复杂的环境中也能稳定吸附在表面并维持其活性[57].

2 基于界面生物修饰的纳米结构的主要功能

2.1 多肽/蛋白质改善纳米颗粒的生物稳定性

多肽和蛋白质能够抑制纳米粒子团聚. 由于尺寸小、表面能大，纳米粒子处于能量不稳定状态，非常容易发生团聚. 纳米粒子的稳定性与其细胞毒性、生物分布和药代动力学息息相关[58]. 团聚一般会导致纳米粒子的细胞毒性增大，在体内的循环时间减短，并且会影响基于纳米粒子载药或成像体系的有效浓度，从而会限制纳米粒子在生物医药领域中的应用. 因此合成稳定、分散性好的纳米粒子是其使用的前提条件. 多肽易于合成，并且可以通过氨基酸序列来调控多肽的亲疏水性和带电情况，因此可以用来调控纳米结构的表面性质，以增加其稳定性. 带有半胱氨酸的多肽可以与金、银等金属形成共价键，经常用来稳定金、银纳米粒子和含金属的量子点等[59~63]. Fernig课题组[60]研究了多肽CALNN和一系列不同长度、不同疏水性和电荷性质的多肽对金纳米粒子稳定性的影响，实验结果表明CALNN包裹后的金纳米粒子稳定性大大提高，能在碱性、中性和微酸性的溶液中稳定存在，并且多肽稳定纳米粒子的能力不但与其长度、亲疏水性、电荷有关，还与氨基酸顺序相关. 蛋白质也能够起到稳定纳米粒子的作用，常用的蛋白质有牛血清白蛋白(BSA)和人血清白蛋白(HSA)，当纳米粒子溶液中加入一定比例的蛋白质时，纳米粒子的分散性会显著提高[64,65].

多肽和蛋白质能够增加纳米颗粒的生物稳定性. 纳米颗粒用于生物体内，还应考虑生物体免疫系统对外来物的清除作用，并且纳米粒子一旦被引入生物体液中，大量的多肽和蛋白质就会吸附在其表面. 吸附的蛋白质被分为两类：一类叫调理素(opsonins)，比如免疫球蛋白G(IgG)[66]，它能使纳米粒子被网状

内皮系统(reticuloendothelial systems)识别，缩短纳米粒子的血液循环时间；另一类叫异调理素(dysopsonins)，比如白蛋白(albumin)[67]，它能够防止纳米粒子被快速清除，增加其血液循环时间[68]. 通常会用PEG修饰纳米粒子来减少其表面的蛋白质吸附，增加体内循环时间. PEG能够中和表面电荷，增加表面亲水性，并提供一定的空间位阻，相当于为纳米粒子提供了保护层，能够减少蛋白质的吸附，但同时也会减少纳米粒子与目标组织和细胞的相互作用和细胞摄入量[69]. 另一种方法是通过多肽或蛋白质来减少调理素在纳米粒子表面的吸附[70]. Lin课题组[71]预先在聚合物纳米颗粒外围包裹BSA蛋白，使其外围预先形成BSA蛋白层，结果表明BSA包裹的纳米颗粒在血液中的循环时间增加，表面的血浆蛋白非特异性吸附减少，纳米颗粒的细胞毒性也大大降低. 除了上述两种方法，还可以通过CD47蛋白及其模拟短肽帮助纳米结构逃避免疫清除，以增加纳米结构的生物稳定性. CD47又称整合素相关蛋白，它与巨噬细胞表面SIRPα结合后可以使细胞免受巨噬细胞的吞噬. Discher课题组[72]通过模拟设计了一条含有21个氨基酸的CD47模拟肽(Self peptide)，并通过Streptavidin和Biotin强相互作用在聚苯乙烯纳米颗粒表面分别连接CD47蛋白和模拟肽，结果表明CD47和模拟肽都能够阻碍纳米颗粒被巨噬细胞清除，增加了纳米颗粒在体内的循环时间，从而增加了纳米颗粒在肿瘤部位的富集(图1).

2.2 基于多肽/蛋白质修饰的纳米颗粒跨膜效应

纳米粒子在大部分生物医学领域的应用中都需要进入细胞核、线粒体等特定细胞器中发挥其功能，这就需要纳米粒子能够跨越细胞膜进入到细胞内. 研究表明，纳米粒子的跨膜能力与其尺寸[73~76]、形状[73,75]、电荷[77,78]等有关，可以通过调控上述参数来增加纳米粒子的入胞. 目前最常用的增加纳米粒子入胞的手段是在其表面修饰穿膜肽(cell-penetrating peptides, CPPs)[79~85]. 穿膜肽是一类具有细胞膜穿透能力的短肽，一般由9~30个氨基酸构成，富含精氨酸和赖氨酸等碱性氨基酸残基. 根据物理化学性质的不同，穿膜肽可分为三类：阳离子型、两亲型、疏水型. 阳离子型穿膜肽带有高净正电荷，包括TAT及其衍生肽、多聚精氨酸等；两亲型穿膜肽包含亲水区和疏水区，除了含有精氨酸和赖氨酸以外，还富含缬氨酸、亮氨酸、异亮氨酸和丙氨酸等疏水氨基酸；疏水型穿膜肽主要以疏水氨基酸为主，带电荷量少[86]. 表面修饰穿膜肽能够赋予纳米结构新的界面功能，增大纳米结构的细胞摄入量，改善其生物分布，进而会增强纳米粒子载药体系和成像体系的治疗和成像效果，例如，在8 nm的银纳米粒子表面通过Ag–S键连接上穿膜肽TAT后，银纳米粒子的入胞量显著增加，其肿瘤细胞杀伤力也提高了20多倍(图2)[87]. 但是穿膜肽缺乏特异性，几乎能够被所有细胞摄取，因此有明显的毒副作用，这种非特异性穿透限制了穿膜肽的体内应用. 研究者发展了以下方法来提高穿膜肽的靶向性. 第1种方法是利用靶向部位特异性表达肽链内切酶的特点定点释放穿膜肽，比如基质金属蛋白酶(MMP)，它是一个肽链内切酶家族，与肿瘤的生长、转移和血管生成息息相关，与正常组织相比，人体内肿瘤组织中的MMP显著上调[88]. 具体原理是：

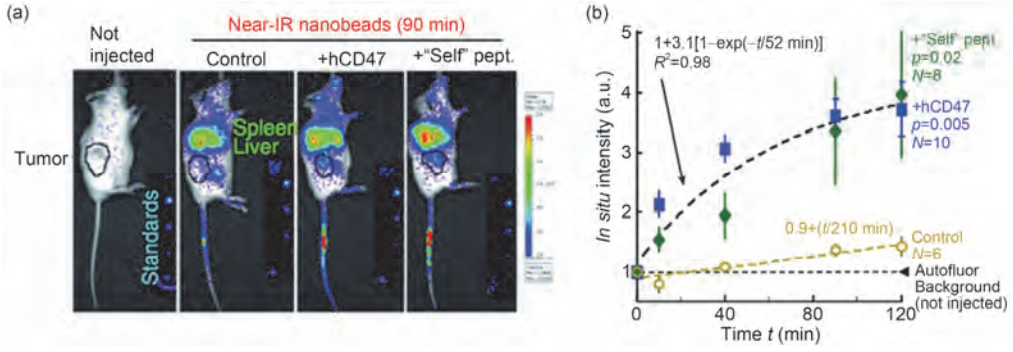

图 1 hCD47 和模拟肽增加了纳米粒子的体内循环时间进而增加了其在肿瘤部位的富集. (a) 小鼠尾部注射连接hCD47 蛋白或Self peptide的纳米球(NPs)后的近红外成像. (b) 肿瘤部位的荧光强度[72]

Figure 1 hCD47 and Self peptides inhibited phagocytic clearance and enhanced delivery of nanoparticles. (a) Near-infrared imaging of mice injected with control NPs, those coated with human CD47, or a CD47-derived peptide. (b) Tumour fluorescence intensity[72]

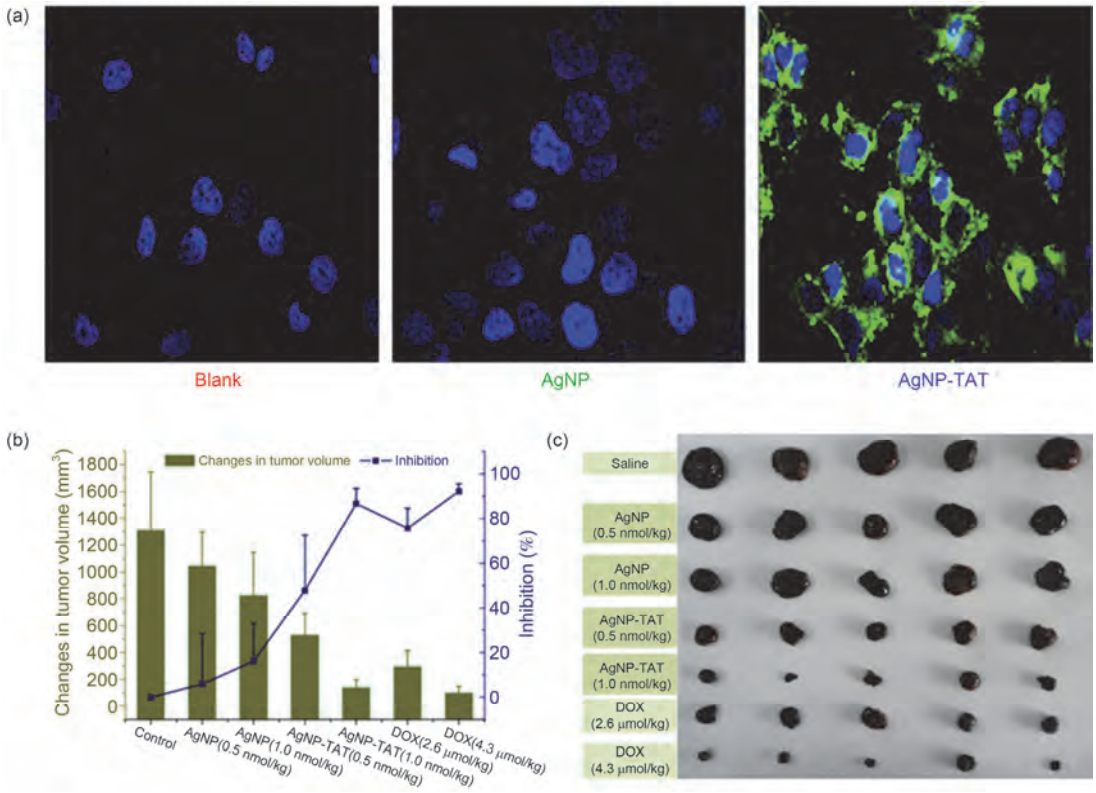

图 2 穿膜肽TAT增加了纳米粒子的入胞量, 进而增加其肿瘤治疗效果. (a) TAT增加了银纳米粒子的细胞摄入量. (b), (c) TAT增加了银纳米粒子对肿瘤的杀伤力[87]

Figure 2 TAT enhanced cellular uptake of nanoparticles and inhibition rate of tumor growth. (a) TAT enhanced the cellular uptake of AgNP. (b), (c) TAT-particle accumulation in tumours enables a reduction in tumour growth[87]

在纳米粒子表面修饰穿膜肽, 然后利用MMP可切断的氨基酸序列作为连接段(linker), 使穿膜肽与另一条与之带相反电荷的多肽(blocker)共价连接, blocker通过静电作用使穿膜肽暂时丧失跨膜能力, 当纳米粒子到达肿瘤部位后, MMP的酶切作用会切断linker使穿膜肽与blocker分离开, 穿膜肽恢复其跨膜能力, 从而达到靶向的目的[89~91]. Tsien课题组[89]在包裹了药物的聚合物纳米粒子表面连接上能够靶向低密度脂蛋白质受体相关蛋白-1(LRP1)的多肽angiopep-2和穿膜肽八聚精氨酸(R8), 并通过MMP的酶切序列PLGLAG使R8与八聚谷氨酸(E8)共价连接, 构筑了MMP可激活的穿膜体系(ACP). Angiopep-2的靶向性加上ACP的靶向性赋予纳米粒子药物递送系统双重靶向性, 增加了纳米粒子在肿瘤部位的富集和药物疗效(图3). 第2种方法是通过与抗体联用增加穿膜肽的靶向性, 例如, 研究者利用脑部毛细血管内皮细胞的转铁蛋白质受体表达量很高这一特点在纳米粒子上同时连接上转铁蛋白质和TAT, Penetratin或多聚精氨酸等穿膜肽用于脑部肿瘤的成像和治疗. 转铁蛋白能够提高纳米粒子对脑部的靶向性, 而穿膜肽又能帮助纳米粒子穿过血脑屏障, 从而增加了成像和治疗的效果[92,93].

2.3 纳米生物结构的靶向性及其在生物检测领域的应用

纳米颗粒的尺寸小、比表面积大, 药物承载率高, 并且可以通过表面修饰增加其生物相容性和体内循环时间, 因此其在药物递送方面有很好的应用前景. 同时纳米粒子通过自身的光学、磁学、热学和声学特性以及作为造影剂的载体, 目前已被广泛应用于光学成像、核磁成像(MRI)、超声成像(US)、计算机断层成像技术(CT)、正电子放射断层造影术(PET)和单光子发射计算机断层扫描(SPECT)成像等临床影像技术中[10~12,94]. 纳米粒子递送系统和成像系统中都要考虑的一个核心问题就是靶向性. 虽然对于实体瘤组织, 纳米粒子可以通过高通透性和滞留效应

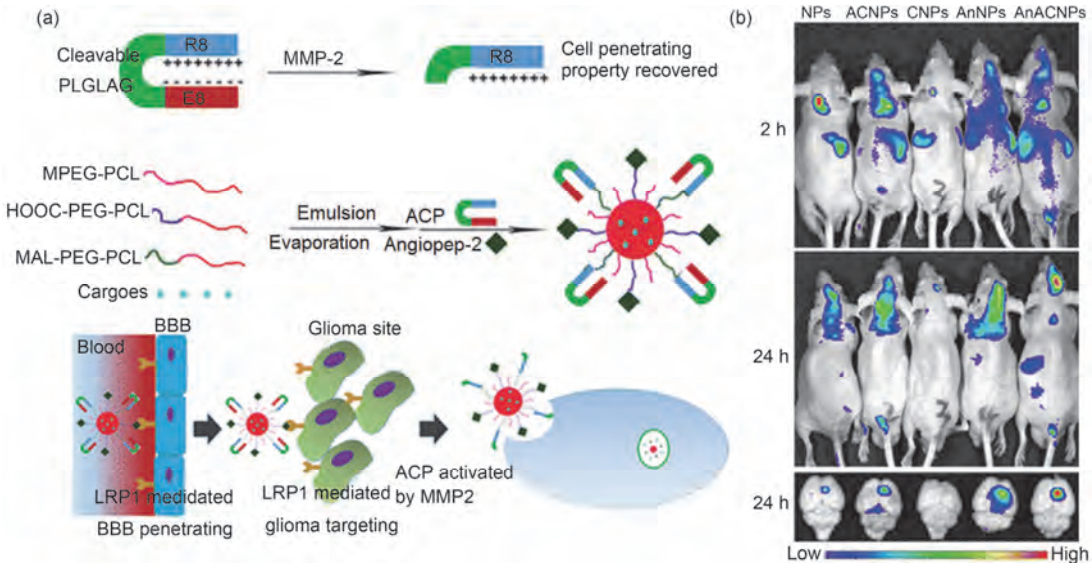

图 3 MMP可激活的穿膜体系和angiopep-2赋予纳米粒子双重靶向性. (a) 胶质瘤的双靶向载药体系构建示意图. (b) 小鼠使用具有不同靶向能力的、包载了荧光染料的纳米粒子后的活体成像和脑部离体成像[89]

Figure 3 Angiopep-2 and activatable cell-penetrating peptide dual-functionalized nanoparticles for systemic glioma-targeting delivery. (a) Elucidation of the dual-targeting delivery system for glioma. (b) *In vivo* imaging of whole body and *ex vivo* imaging of brain from mice that treated with different DiR-loaded formulations[89]

(EPR效应)被动靶向到肿瘤组织中[95~97], 但是目前EPR效应在药物临床使用时是否能起到靶向作用仍存在不确定性, 并且不同个体或者同一个体的不同时期, 人的脉管系统有显著差异, 所以主动靶向必不可少[96,98,99]. 目前实现主动靶向最常用的方法是在纳米结构表面结合上能够靶向肿瘤细胞和肿瘤组织血管内皮细胞的多肽和蛋白质[100~104]. 靶向肿瘤细胞和肿瘤内皮细胞常用的靶点分别有: 转铁蛋白(transferrin)、叶酸(folate)、表皮生长因子受体(epidermal growth factor receptor)、糖蛋白受体(glycoproteins receptors)和血管内皮生长因子(VEGF)、$\alpha_v\beta_3$整合素、血管细胞黏附分子-1(VCAM-1)、MMP[98]. Cheon课题组[105]合成了锰参杂的磁性氧化铁纳米粒子(MnMEIO), 然后在纳米粒子表面包裹BSA, 通过BSA上的氨基与交联剂3-(2-吡啶二巯基)丙酸*N*-羟基琥珀酰亚胺酯(SPDP)反应, 在纳米粒子表面引入二硫键, 最后共价连接上靶向$\alpha_v\beta_3$整合素的多肽RGD和具有基因治疗作用的小干扰RNA(SiRNA), 实现了乳腺癌靶向给药和多模成像一体化(图4). RGD不仅使纳米粒子能够靶向乳腺癌肿瘤细胞, 而且增加了纳米粒子的入胞, 进而增加药物SiRNA的入胞. 梁兴杰课题组[106]在纳米胶束上共价连接上靶向神经纤毛蛋白1(neuropilin-1)的多肽CRGDK, CRGDK显著提高了纳米胶束载药体系在肿瘤部位的富集和肿瘤治疗效果. 本课题组筛选出了一条能够靶向趋化因子受体CXCR4用以阻断CXCL12/CXCR4趋化轴的多肽E5[106], E5与纳米胶束非共价结合后提高了纳米胶束载药体系的靶向性和药物疗效[107]. 除了上述介绍的靶向生物标记物外, 还可以通过靶向肿瘤微环境来提高纳米载药和成像体系的靶向性, 比如低pH插入肽(pH low insertion peptide, pHLIP), 这类多肽由侧翼序列和跨膜序列组成, 侧翼序列多为质子化氨基酸残基构成, 跨膜序列一般为疏水氨基酸, 在酸性条件下可形成螺旋结构嵌入细胞膜, 这类多肽可作为靶向分子靶向肿瘤酸性微环境[108,109]. Reshetnyak课题组[103]在金纳米粒子上共价结合上低pH插入肽WT-pHLIP, 实验结果表明WT-pHLIP增加了纳米粒子在肿瘤细胞膜表面的富集, 进而增加了对肿瘤细胞的辐射损伤.

纳米颗粒具有独特的光学、磁学和电学等性质, 而生物分子具有选择性和特异性, 包括DNA序列的特异性识别、抗原-抗体的特异性识别, 二者的结合可以用于生物信号的检测和放大. 目前, 多肽/蛋白质-纳米颗粒复合结构已广泛应用于DNA[110,111]、蛋白质[112~114]、病原体[115,116]和循环肿瘤细胞(CTC)[118,119]的检测, 研究最多的是金纳米粒子. 多肽/蛋白质-纳

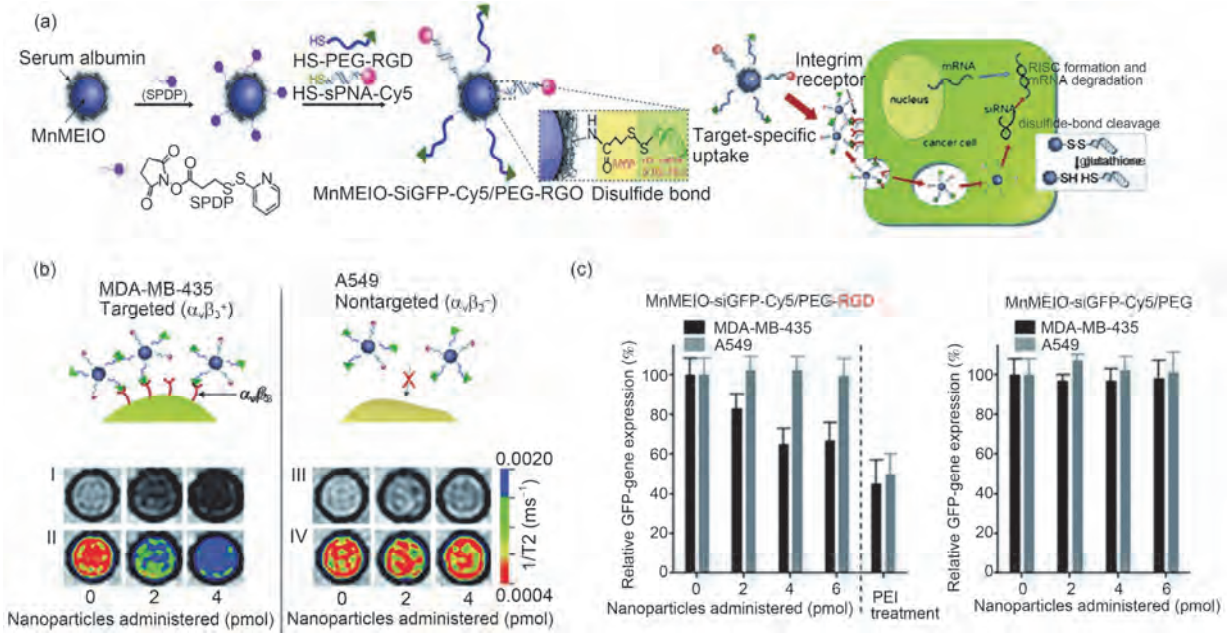

图 4 磁性纳米粒子与靶向$\alpha_v\beta_3$整合素的多肽RGD结合后用于肿瘤成像和治疗. (a) 构建纳米粒子MnMEIO-siGFP-Cy5/PEG-RGD的示意图. (b) RGD纳米粒子通过与$\alpha_v\beta_3$相互作用能够靶向MDA-MB-435 细胞. (c) RGD能够增加SiRNA的疗效[105]

Figure 4 RGD-modified magnetic nanoparticles for simultaneous molecular imaging and siRNA delivery. (a) Schematic illustration of multimodal MnMEIO-siGFP-Cy5/PEG-RGD. (b) RGD-conjugated nanoprobes bind specifically to the $\alpha_v\beta_3$-positive cells (MDA-MB-435). (c) RGD increased the efficacy of SiRNA[105]

米颗粒复合结构在生物检测中的应用主要有以下4个方向. (1) 富集检测物. 许多目标物的浓度过低会给检测带来很大的困难, 比如CTC, 它在血液中的浓度极低, CTC 研究的核心在于能否在复杂环境中高效地富集CTC. 而在纳米颗粒表面修饰针对不同目标物的抗体和多肽就能起到富集检测物的目的. 最常用的是修饰了抗体的磁性纳米颗粒. 美国食品和药品监督管理局(FDA)通过的用于乳腺癌细胞检测的方法CellSearch系统就是利用修饰了上皮黏附因子(EpCAM)抗体的磁性纳米颗粒对血液中的CTC进行富集, 之后利用免疫荧光法进行计数[119]. 本课题组[120]针对EpCAM蛋白, 筛选出了与之具有较强亲和力的多肽Pep10, 200 nm磁性纳米颗粒表面通过Biotin-Streptavidin相互作用连接Pep10后用于CTC的捕获, 捕获效率能够达到EpCAM抗体的90%(图5). 目前市面上有很多不同尺寸的免疫磁珠出售, 主要用于细胞、蛋白质的分离和纯化. (2) 多肽/蛋白质-纳米颗粒复合结构自身的特性改变直接作为检测信号. 例如, 金、银等贵金属纳米粒子具有很强的局域表面等离激元共振效应, 共振吸收光谱峰值处的吸收波长和吸收强度不但与该材料的组成、尺寸等微观结构特性有关, 还对周围介质和粒子间距离极其敏感, 因此可以作为基于光学信号的生物传感器. 目前很多研究工作都是基于金纳米粒子团聚的比色检测[114,121~124]. Chang课题组[125]将这种传感原理用于凝血酶(thrombin)的检测. 在金纳米粒子溶液中加入过量的纤维蛋白质原(fibrinogen), fibrinogen通过静电和疏水作用吸附在金粒子表面, 当溶液中加入凝血酶时, 溶液中和吸附在金粒子表面的纤维蛋白原会聚合成不溶性的纤维蛋白, 从而引起金纳米粒子的聚集, 通过测量溶液的上清在最大吸收波长532 nm处吸收强度的降低来计算凝血酶的浓度, 检测线能够达到0.04 pmol/L(图6). (3) 多肽/蛋白质-纳米颗粒复合结构可以作为信号分子的载体用于生物检测. Tan课题组[111,116,118]利用生物分子修饰的二氧化硅纳米粒子包载金属有机荧光分子用于DNA、细菌和癌细胞的检测, 包裹于纳米粒子内的荧光分子具有更好的光稳定性. 有研究者基于酶联免疫吸附测定(ELISA)的原理, 同时在纳米粒子表面修饰二抗和辣根过氧化物酶(HRP)用于蛋白质检测[126,127]. (4) 多肽/蛋白质-纳米颗粒复合结构可以作为信号放大系统用于检测[128~133], 例如, 蛋白质修饰的磁性纳米粒子和金纳

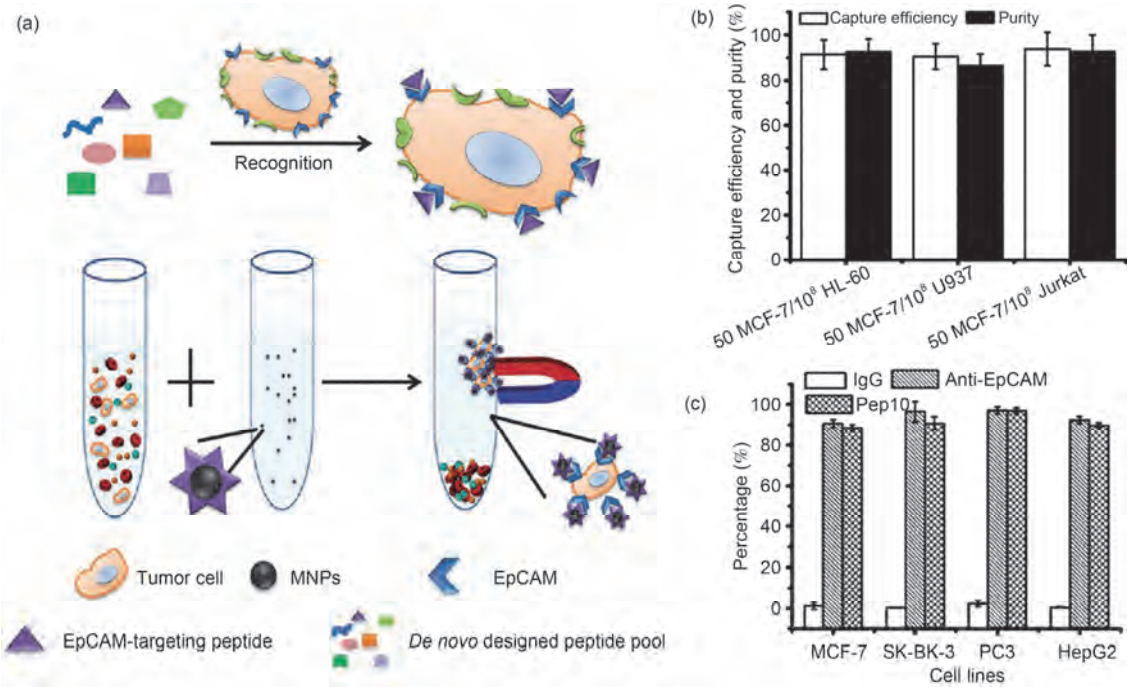

图 5 磁性纳米粒子修饰EpCAM靶向肽后用于CTC捕获. (a) 基于磁性纳米粒子和EpCAM靶向肽构建CTC捕获体系示意图. (b) Pep10的捕获效率和特异性. (c) Pep10 和EpCAM抗体的捕获效率的对比[120]

Figure 5 Magnetic nanoparticles modified EpCAM-targeting peptide for CTC capture. (a) Schematic of a CTC capture system based on magnetic nanoparticles and EpCAM-targeting peptides. (b) Capture efficiency and capture purity of Pep10. (c) Capture efficiency of Pep10 and EpCAM antibody[120]

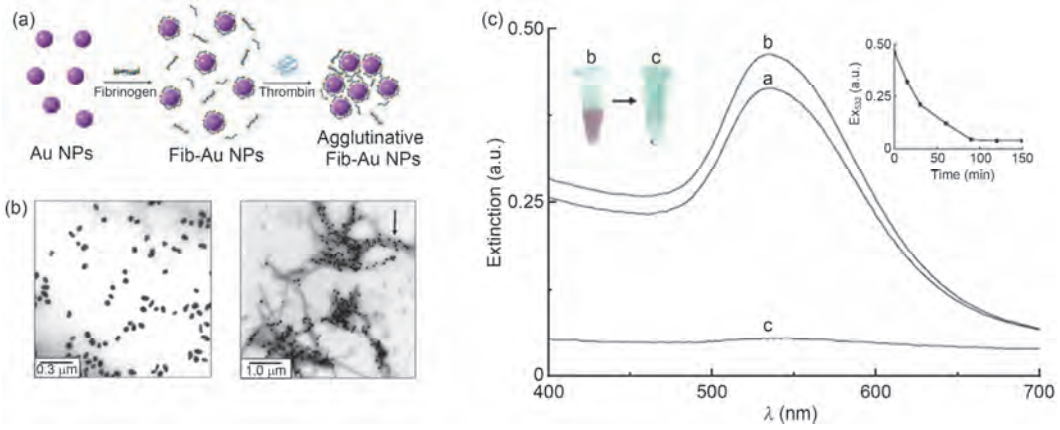

图 6 基于金纳米粒子的比色法用于检测凝血酶. (a) 基于金纳米粒子的凝血酶传感器示意图. (b) 在纤维蛋白原和金纳米粒子的混合溶液中加入凝血酶, 金纳米粒子发生聚集. (c) 在纤维蛋白原和金纳米粒子的混合溶液中加入 100 pmol/L的凝血酶, 离心后的上清在最大吸收波长处的吸收强度下降[125]

Figure 6 Label-free colorimetric detection of picomolar thrombin using a gold nanoparticle-based assay. (a) Schematic of a thrombin sensor based on gold nanoparticles. (b) 100 pmol/L thrombin made AuNPs aggregate with the presence of fibrinogen. (c) The absorption intensity of AuNPs in supernatant at the maximum absorption wavelength reduced after aggregation[125]

米粒子可以用于增强表面等离激元共振(SPR)的信号强度[129,130]. 首先在SPR芯片上连接能够特异性识别目标物的抗体(一抗), 当目标物接触芯片时会通过抗原-抗体特异性识别吸附在芯片表面, 在体系中再加入修饰了二抗的纳米粒子, 一抗-检测物-二抗三者形成“三明治”结构. 纳米粒子自身的质量和金属离子表面的LSPR效应都会增加SPR的信号强度. 金、银等金属纳米粒子由于大的比表面积和LSPR效应, 表面

修饰生物分子后被广泛用作增强基底应用于表面增强拉曼光谱分析(SERS), 这也是基于多肽/蛋白质-纳米颗粒复合结构对检测信号的放大作用[117,134].

2.4 纳米生物结构在应用中面临的挑战

纳米生物结构多应用于体内或离体的生物体液中, 如前文所述, 纳米生物结构一旦引入生物体液中, 其表面会立即与蛋白质、核酸等生物分子发生相互作用, 形成新的生物界面. 纳米材料表面吸附的蛋白层被命名为蛋白冠(protein corona). 蛋白冠一直处于动态平衡状态, 纳米结构与生物体液一旦接触, 其表面首先会吸附高丰度的蛋白质, 但这类蛋白质与纳米结构表面亲和力低, 很快会被丰度相对较低而亲和力高的蛋白质所取代, 后者与纳米结构结合紧密, 不容易被周围蛋白质所取代, 形成“硬质”蛋白冠(hard corona), “硬质”蛋白冠外围则为结合松散、与周围环境交换速度较快的“软质”蛋白冠(soft corona)[135]. 蛋白冠的形成会覆盖纳米生物结构原有的界面性质, 不但对纳米生物结构在体内的循环时间、生物分布和生物相容性有重大影响[136,137], 同时还会影响其表面生物分子的活性和功能[138,139]. Dawson课题组[138]在二氧化硅纳米粒子上共价修饰上转铁蛋白(transferrin), 功能化的纳米粒子在磷酸缓冲液(PBS)中能够保持其靶向性, 但是在胎牛血清(FBS)和人血清中, 由于蛋白冠的形成, 转铁蛋白的靶向功能丧失. 所以, 除了在构建纳米生物结构时考虑生物分子在表面的结构和朝向, 还应考虑在使用过程中周围环境对生物分子稳定性和活性的影响. 研究蛋白质与纳米结构界面的相互作用能够为纳米生物结构的构建和其在复杂环境中发挥正常功能提供基础性指导. 研究者利用表面等离激元共振(SPR)、等温滴定量热(ITC)、石英晶体微天平(QCM)和荧光相关光谱(FCS)等方法研究蛋白质与纳米界面的亲和力及多肽和蛋白质的吸附动力学、热力学[140,141], 利用傅里叶变换红外光谱(FTIR)、圆二色谱(CD)等方法研究蛋白质与纳米界面相互作用后结构的变化[135,142], 利用同步辐射研究多肽和蛋白与界面的结合模式[143], 利用扫描隧道显微镜(STM)从分子水平上探究氨基酸和多肽在表面的组装行为[144~148]. 此外, 二次谐波产生(SHG)[149]和频振动光谱(SFG)[150]等界面敏感技术也广泛用于多肽和蛋白与界面的相互作用研究. 本课题组[144~148,151,152]利用STM研究了一系列淀粉样多肽和模型肽在疏水表面的组装行为, 结果表明单个氨基酸突变对多肽在表面的组装有很大影响, 多肽在表面的组装稳定性可以通过氨基酸侧链的亲疏水性、磷酸化和糖基化等性质调控. 目前在纳米生物界面研究中已取得了一些成果, 但是对于生物分子与纳米界面的相互作用以及界面处的生物分子结构、生物组分和分布仍缺乏全面的认识, 对纳米生物界面进行人为调控仍然存在很大挑战.

3 总结

表面修饰能够赋予纳米结构新的性质和功能, 目前, 基于多肽/蛋白质表面修饰的纳米结构已广泛用于药物递送、成像和检测等生物医药领域. 多肽和蛋白质可通过共价和非共价两种方式与纳米材料结合构筑具有特定功能的纳米生物结构, 但是目前在精确调控生物分子在表面的取向和结构等方面仍存在很大困难, 同时纳米生物结构在使用过程中还会面临周围环境对其表面性质的影响. 纳米生物界面的结构和性质事关纳米生物结构的构筑及其使用过程中的方方面面, 对它的研究不仅有助于发展新的纳米生物结构构建方法, 还有助于我们理解其在使用过程中与生物体系的相互作用及蛋白冠的组成和演变, 最终实现精确调控和利用纳米生物界面的目的.

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Summary for “基于多肽和蛋白质分子的纳米生物界面效应及其应用研究”

Peptide-/protein-mediated nano-bio interface and its applications

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Surface modification can impart nanostructures new interface properties. In this review, we summarize the representative surface modification methods of nanoparticles with peptides and proteins. The biomolecules can be conjugated with nanoparticles by noncovalent and covalent coupling. Both of these approaches have strengths and limitations. Physical adsorption is the most direct and simplest noncovalent method but peptides and proteins adsorbed on solid surface always loss their native structures and biological activity and the behavior of peptides and proteins on surface is still difficult to regulate precisely. A popular noncovalent conjugation with stability and selectivity is via biotin-streptavidin interaction. Covalent attachment is more stable and selective than noncovalent methods. The stability of covalent biomolecule-nanostructure conjugates makes this strategy useful for applications in biological media with other interfering species. 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC)/*N*-hydroxysuccinimide (NHS) strategy is widely used for covalent bioconjugation with well-established protocol, but it could lead to hydrolysis problem, crosslinking and uncontrolled heterogeneous orientation. The click chemistry is another covalent strategy with higher selectivity and yield, while it takes an extended reaction time. In addition to considering stability, selectivity, reaction time, and yield mentioned above, covalent bioconjugation should proceed in mild condition and the reagents required should have no interference on properties of biomolecules and nanostructures. In recent years substantial progress has been made in the formation of bionanoconjugates, but there is still no completely reliable approach for biomolecule-nanosturcture conjugation. Heretofore fine-tuning the structure and orientation of peptides and proteins on surface has been challenging.

The efforts on improving biostability, biodistribution and targeting of nanoparticles with peptides and proteins functionalization are introduced. Nanostructures are often used within body in biomedical applications, so they must overcome an ensemble of biological obstacles to perform their function. First they may suffer from clearance from the body’s immune system. Even though they can escape from clearance, they are required to arrive their correct battlefield. What’s more, nanostructures need to cross cell membrane barriers and reach specific organelles such as nucleus and mitochondria to function in most cases. Peptide- and protein-nanostructure conjugation has been emerged as a useful tool to address these problems mentioned above. They could provide nanostrctures protection from phagocytic clearance and promote persistent circulation. Nanostructures can penetrate cell membrane easily with cell-penetrating peptide modification. The specific recognition of biological molecules imparts nanostructures targeting ability. In addition, the applications in fields such as diagnostics are introduced based on antigen-antibody specific recognition.

The problems that bionanoconjugates for medical applications may encounter in use are presented briefly. Once contacting with biological matrices, nanostructures will be immediately coated by proteins, forming a protein corona. This protein layer could make significant changes in nanostructure properties such as size, surface charge and even the bioactivity of the peptides and proteins conjugated on its surface, which provides nanostructure with a new biological identity. Some previous results suggested that protein-functionalized nanoparticles lost their targeting capabilities when a protein corona adsorbed on the surface. In short, not only should bioconjugation methods be picked carefully depending on the goals, but also the effects of the complex use environment on bionanoconjugates should be taken into consideration.

This review is intended to help researchers get an idea of the progress and dilemma of bionanoconjugate construction and its applications, and provide some inspiration for design and synthesis of peptide and protein-modified nanostructures.

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