特约评述

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仿生分区室固定化多酶体系

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摘要：仿生分区室固定化多酶偶联是体外合成生物学的前沿技术，目的是实现多酶分区室固定化和反应的时空分离。与简单共固定化不同，仿生分区室固定化技术通过控制酶在载体上的空间分布，形成底物通道促进中间产物传递，并提高串联或偶联反应的系统稳定性、产率和产物纯度。本文综述了近年来仿生分区室固定化多酶体系的进展，包括金属有机框架（MOF）、聚合物囊泡和聚合物胶囊等固定化策略。MOF具有结构可控、功能易调控等优点，采用分级多孔、MOF-on-MOF和多种MOF组合等仿生策略构建分区室微反应器，可实现高效的体外多酶偶联催化反应。聚合物囊泡的膜结构可模拟天然磷脂双分子层，将多个小囊泡包封到大囊泡形成“囊泡中囊泡”模仿细胞器分区室固定化酶。聚合物胶囊是通过模板法形成的核壳纳米球体结构，结构稳定性优异，进一步通过层层自组装能够形成多层核壳结构实现分区室固定化。将来，微流控等技术与仿生分区室固定化多酶技术融合，将促进体外合成生物学和绿色生物制造等领域的发展。

关键词：多酶偶联；仿生分区室；固定化酶；金属有机框架；聚合物囊泡；聚合物胶囊

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Biomimetic compartmentalization immobilization of multi-enzyme system

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Abstract: Biomimetic compartmentalization immobilization of multi-enzyme system is a frontier for *in vitro* synthetic biology, focusing on the spatial and temporal separation of reactions. Compared with simple co-immobilization, biomimetic compartmentalization immobilization can form substrate channels and promote the transmission of intermediates for sequential or coupling reaction. By controlling the relative positions of the enzymes on carriers, this method improves system stability, productivity, as well as purity of product. In this review, we summarized the recent advances of carriers for biomimetic compartmentalization immobilization of multi-enzyme

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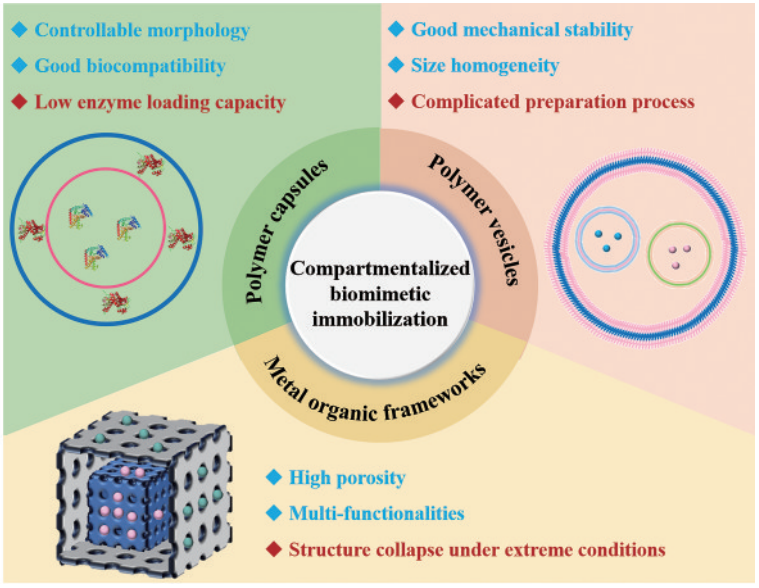
systems, including metal-organic frameworks (MOFs), polymer vesicles and polymer capsules. Metal-organic frameworks (MOFs) are porous coordination materials which are composed of metal ions as nodes and organic linkers. MOFs possess unique characteristics including high porosity, large specific surface area and tunable structure, which are suitable for multi-enzyme systems. The strategies involving the hierarchically porous MOFs, MOF-on-MOF and multi-MOF combinations construct compartmentalized environments for efficient catalytic reactions *in vitro*. Polymer vesicles are hollow nanostructures composed of amphiphilic block copolymers. The membrane structure of polymer vesicles, similar to the natural phospholipid bilayers, has good mechanical stability and biocompatibility for protecting enzyme molecules, and provides unique microenvironment for sequential reactions. Multiple small vesicles were encapsulated into the larger vesicles to form a “vesicle-in-vesicle” by mimicking the structure of cellular organelles. Polymer capsules with a core-shell spherical nanostructure are formed by the templating method, and have structural stability and excellent shape controllability. Multilayered core-shell structures created by layer-by-layer self-assembly are applied for compartmentalized immobilization of multi-enzyme. In the future, the integration of microfluidic technologies with biomimetic compartmentalization immobilization of multi-enzyme is expected to provide highly efficient and stable multi-enzyme catalytic systems for *in vitro* synthetic biology and green biomanufacturing.



Keywords: multi-enzyme coupling; compartmentalized biomimetic; immobilized enzyme; metal-organic frameworks; polymer vesicles; polymer capsules

体外多酶反应网络结合了体外生物催化的优势以及体内多步途径的强大功能，可用于设计自然界中未发现的生物系统，是体外合成生物学的重要研究方向。体外多酶偶联催化体系[1-4]是指多种不同催化功能的生物酶分子在细胞外进行组合，模拟细胞内的酶催化过程[5-6]，在体外环境中实现复杂化合物的合成或分解。与细胞催化相比，具有反应速度快[7]、选择性好以及产量高等优势。目前，体外多酶催化已广泛应用于食品加工[8-9]、生物传感[2, 10]、生物医学[11-12]以及化学合成[13-16]等方面。

在空间上划分出紧密联系的区室，实现分区室固定化多酶，调控各个酶催化反应的微环境，是提高体外合成生物学体系催化效率的重要策略[17-20]。自20世纪后半叶以来，科学家们开始模仿细胞器的形状、组成及功能[21-25]，开发体外仿生

多酶偶联体系，以实现更加高效、环保的体外合成过程。然而，多数仿生多酶偶联体系是将多酶随机固定在同一个载体上，这种简单共固定策略无法控制酶的空间分布，不适合需要精确控制反应顺序和速率的多步酶反应，无法模拟细胞区室中特定微环境下发生的催化反应。仿生分区室固定化多酶偶联是在载体材料上创建分隔的微环境[26]，将多酶分隔在不同区室中，模仿细胞内生物酶的空间分布，其优势在于能够实现中间产物的顺序传递，降低外界环境对酶的干扰，避免酶失活，实现高效底物转化[27-28]和产品合成[29]。

仿生分区室固定化多酶的载体材料[30]包括金属有机框架[31]、聚合物囊泡[32-34]、聚合物胶囊[35]等，选择合适的载体材料能有效提高酶活性以及催化反应的稳定性，同时通过合理的载体设计构建仿生区室化微环境，将多酶固定在不同区域实现空间隔离和反应条件优化，对提高多酶偶联反应的效率、选择性以及产率具有重要意义。

1 金属有机框架材料

金属有机框架（metal-organic framework，MOF）是由金属离子与有机配体组成的晶体材料，良好的结构可调控性及表面性能使其能够实现仿生分区室固定化酶（表1），为多酶分子创造类似细胞器中的隔间[36]。MOF的孔道结构和拓扑结构多种多样，能够以表面吸附、共价键合，孔包封和共沉淀的方式与酶分子结合。如图1所示，MOF分区室固定化多酶通常是通过分级多孔MOFs、MOF-on-MOF以及多MOFs组合分区来实现的，使其有序、高效、可控地进行偶联反应，有效模拟了细胞内复杂的催化过程[50]。

表1 MOFs分区固定多酶

Table 1 MOFs compartmentalized immobilized multi-enzyme

固定化策略	MOFs	酶	稳定性	参考文献
分级多孔MOFs分区	PCN-888	GOx和HRP	4次循环后，GOx&HRP@PCN-888活性保持不变	[36]
分级多孔MOFs分区	PCN-333(Al)	HRP和胆固醇氧化酶（ChOx）	4 ℃下保存20 d后，GOx&HRP@PCN-333仍可检测到50%酶活	[37]
分级多孔MOFs分区	PCN-333(Al)	超氧化物歧化酶（SOD）和过氧化氢酶（CAT）	SOD&CAT@FNPCN-333在储存7 d后仍可检测到酶活	[38]
MOF-on-MOF	ZIF-8	GOx和HRP	HRP@H-ZIF-8-GOx储存7 d后仍可检测到70%酶活	[39]
MOF-on-MOF	ZIF-8	GOx和HRP	GOx@ZIF-8@HRP@ZIF-8在4 ℃储存10 d后仍可检测到93.96%酶活	[40]
MOF-on-MOF	ZIF-8	GOx和HRP	—	[41]
MOF-on-MOF	Amine-MIL-101 (Cr) and HKUST-1	CA，FDH和GDH	10个循环后，产率达到1077.7%	[42]
MOF-on-MOF	HKUST-1	GOx和HRP	HRP@GOx@HKUST-1@Fe_3O_4重复使用10次后仍可检测到80.6%酶活	[43]
MOF-on-MOF	MOF-74	脂肪酶（CALB）and GOx	5个循环后仍可检测到79.3%酶活	[44]
多MOFs组合	ZIF-90	*N*-乙酰己糖胺-1-激酶（NahK），尿苷二磷酸-*N*-乙酰半乳糖胺焦磷酸酶（GlmU）和多磷酸激酶（PPK）	—	[45]
多MOFs组合	ZIF-8	GOx，HRP和β-Gal	—	[46]
多MOFs组合	ZIF-8	FDH，GDH和NADH	FDH&GDH&NADH/ZIF-8使用12 h后保留50%酶活	[47]
多MOFs组合	UiO-66	HRP和GOx	GOx@MOF-Cs和HRP@MOF-Cs 3个循环后酶活性不变	[48]
多MOFs组合	ZIF- L and MPN	GOx和HRP	GOx-ZIF-L和HRP-ZIF-L在4 ℃保存30 d仍可检测到酶活	[49]

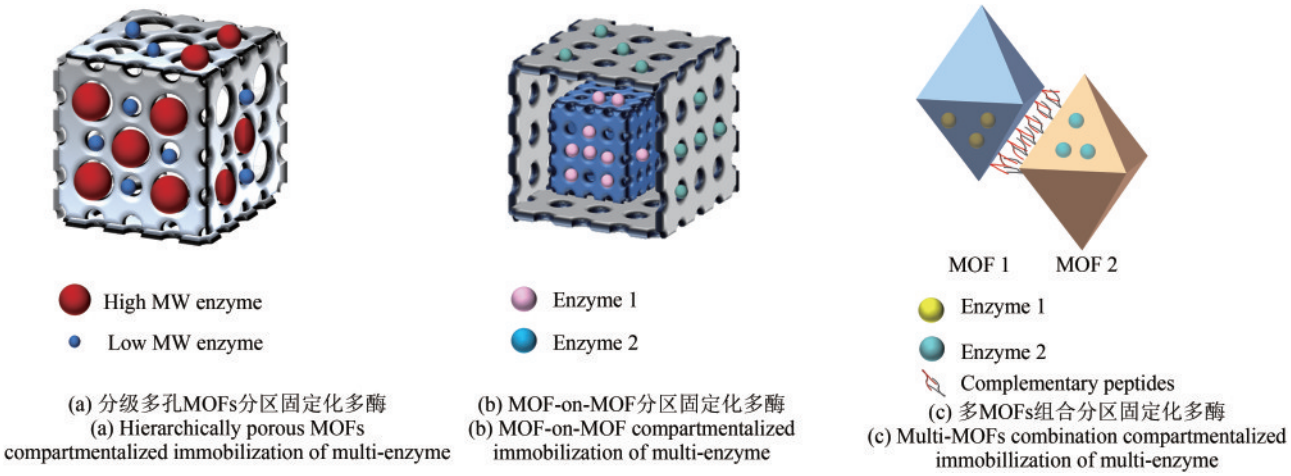


图1 MOFs分区固定化多酶

Fig. 1 Immobilization of multi-enzyme by compartmentalized MOFs

1.1 分级多孔MOFs分区固定策略

MOFs能够通过多种合成策略来调控孔隙大小和形状。将不同大小的酶分子通过孔道吸附的方式先后固定在MOFs不同尺寸的孔道中，实现分区固定。这种方法不仅保留了酶的活性，而且通过控制酶在MOFs中的空间排布，可以提高催化效率和选择性。

PCN-888的孔道空腔呈蜂窝状排列，有6.2 nm、5.0 nm和2.0 nm三种尺寸。Lian等[36]根据酶分子的大小将葡萄糖氧化酶（GOx）固定到PCN-888最大的孔道、辣根过氧化物酶（HRP）固定到5.0 nm的孔道，最小的孔道则作为底物或产物通道，构建了一个分区室固定化多酶偶联体系。该体系展现出了良好的酶催化活性以及抗胰蛋白酶稳定性。但由于该体系是通过π-π作用、疏水相互作用等弱相互作用将酶分子吸附到PCN-888孔道中，重复利用4次后酶分子开始脱落导致体系活性下降。此外，球形MOF PCN-333结构中也含有尺寸分别为4.0 nm和5.5 nm的介孔空腔，Zhou团队[38]将超氧化物歧化酶（SOD，2.8 nm×3.5 nm×4.2 nm，16.3 kDa）和过氧化氢酶（CAT，4.9 nm×4.4 nm×5.6 nm，60 kDa）先后固定到PCN-333中，CAT占据5.5 nm空腔，SOD占据4.0 nm空腔，形成SC@PCN-333纳米器件，用于减轻细胞氧化应激影响。PCN-333在水溶液中能够长时间保护孔道中酶分子生物活性，SC@PCN-333储存7 d后仍可检测到酶活。

1.2 MOF-on-MOF分区固定策略

MOF-on-MOF分区固定是通过各种生长策略（如随机外延生长、有序外延生长等）在第一种MOF的表面上生长第二种MOF，对酶分子进行层层固定，形成多层分区固定，在微观层面上控制酶的分布，避免相互干扰和竞争。体系中的底物和中间体通过孔道扩散，在不同壳层中酶分子的催化作用下，实现串联或者偶联反应。Li等[42]以MIL-101（Cr）为核心，外延生长两层HKUST-1，通过共沉淀的方法固定多酶。将碳酸酐酶（CA）固定在HKUST-1内层，甲酸脱氢酶（FDH）和谷氨酸脱氢酶（GDH）固定在HKUST-1外层。吸附CO_2的MIL-101能够提高体系CO_2浓度，内层的CA将CO_2转化为HCO_3^-，扩散到HKUST-1的外层后在FDH的作用下转化为甲酸盐。固定于外层的GDH则用于实现整个体系NADH的辅酶再生。MIL-101（Cr）和HKUST-1具有良好的化学稳定性，能够保护酶分子不受外界干扰，且采用共沉淀的方法进行酶固定化，使得酶分子不易脱落，经过10个催化循环后，基于辅酶因子的甲酸盐产率能够达到1077.7%。Man等[40]通过共沉淀法，在室温下将GOx和HRP依次封装在多层ZIF-8 MOFs中，以葡萄糖为底物，研究多层ZIF-8 MOFs微反应器的生物催化级联反应。相对于游离的GOx&HRP系统，GOx@ZIF-8@HRP@ZIF-8反应催化效率提高了5.8倍。此外，GOx@ZIF-8@HRP@ZIF-8

在4 ℃储存10 d后仍可检测到93.96%酶活。

通过MOF-on-MOF固定化酶的设计和构建，能够在同一体系中协同进行复杂的串联反应或多步酶催化反应。这种方法优化了酶与底物之间的接触和扩散，提高催化效率以及酶的稳定性。

1.3 多MOFs组合分区固定策略

将多个酶分别固定在MOFs粒子中，并对固定不同酶分子的MOFs粒子通过互补肽、微流控、膜技术等方式进行有序组合，实现多酶分区固定化，中间产物在孔道存在下进行传递，使得多酶偶联催化反应能够连续进行。

Liang等[46]利用多肽接头诱导MOFs超组装的方法，制备了一种反应可控的多酶偶联反应器。首先将GOx、HRP和β-半乳糖苷酶（β-Gal）分别封装于ZIF-8中，再对封装不同酶的MOFs粒子进行互补肽的表面功能化，诱导MOFs粒子自组装形成分区室化多酶偶联体系。此外，改变条件使得互补肽解体可导致MOFs组合分散，从而终止多酶偶联反应，实现对反应过程的可控性。Xu等[48]分别将含有GOx和HRP的聚甲基丙烯酸甲酯（PMMA）水溶液与疏水性的UiO-66-NH_2 NPs十二烷溶液混合，通过剪切均质机在十二烷中制备油包水Pickering乳状液。接着通过溶剂蒸发将PMMA沉积在MOF胶囊（MOF-Cs）内部，得到包封GOx和HRP的MOF-Cs（GOx@MOF-Cs和HRP@MOF-Cs）。MOF-Cs具有保护内部微环境以及通过分隔实现串联反应的能力。因此将GOx@MOF-Cs和HRP@MOF-Cs混合能够得到区室化双酶偶联体系。Zhu等[47]开发了微孔膜与MOFs相结合的多酶偶联系统，将甲酸脱氢酶（FDH）、甲醛脱氢酶（FalDH）以及乙醇脱氢酶（ADH）分别固定在ZIF-8中，接着将包封不同酶的MOFs纳米复合物材料依次固定于渗透膜中，实现多酶偶联反应。膜中固定化酶有序分布的反应效率高于膜中无序分布的反应效率。多MOFs组合分区固定化多酶偶联反应的优势在于对偶联反应条件以及底物分子运输都能进行更好的调控。

2 聚合物

聚合物具有良好的可调控性，广泛的单体来源赋予其多样的化学结构和性质，是制备生物催化微反应器的优良选择[51]（表2）。

根据结构特点和分散介质的不同，聚合物可以分为胶囊、囊泡等。基于嵌段共聚物自组装形成的聚合物囊泡，其制备过程温和，具有稳定性良好的双层膜结构，适用于包封亲水性活性物质。核壳结构的聚合物胶囊，通常采用能够提供良好载体和保护性能的聚合物材料[63-65]，如聚乙烯吡咯烷酮（PVP）、聚乳酸（PLA）等，其结构和形状可控，在酶固定化方面能够为酶分子构象提供稳定的微环境以及物理保护，一般用于提高酶稳定性和可回收性。但由于聚合物胶囊在应用过程中可能会发生解离或破裂，作为纳米反应器的实际应用较少[66]。

2.1 聚合物囊泡

聚合物囊泡[67]是由嵌段共聚物自组装[68]形成的，具有与细胞器膜相似的双层膜结构，生物相容性好，其亲水性的内腔可包封酶分子形成酶促反应场所。由多个囊泡组合形成的聚合物纳米反应器[68-71]尺寸范围广，从几十纳米到几微米，功能类似于细胞器，在空间上不仅能够将反应条件不相容的酶分子分隔开，还能允许体系中小分子底物和产物的扩散[72]。聚合物囊泡的膜厚度、柔韧性以及渗透性可调，同时具有良好的机械性能，其稳定性优于天然磷脂双分子层。因此，由聚合物囊泡形成的仿生分区室化纳米反应器[73]，具有保护酶分子和支持多酶偶联反应连续进行的双重作用。

在多室囊泡构建过程中引入膜蛋白到聚合物囊泡的膜中，使得体系中的底物分子有选择性地通过膜进入微区室反应，既能保证多酶偶联中每步催化反应在不同区室中完成，又能实现区室间物质传递。例如Ces团队等[54]通过液滴相转移（GUV）的方式产生连续的两室囊泡，并将GOx以及HRP分别包封在两个区室形成多酶偶联催化体系，同时在多室囊泡自组装的过程中插入跨膜蛋

表2 聚合物与生物材料分区室固定多酶

Table 2 Polymer and biomaterial compartmentalized immobilized multi-enzyme

材料	酶	稳定性	参考文献
聚合物囊泡[聚甲基丙烯酸酯(PMA)和聚赖氨酸(PLL)]	GOx和HRP	4 ℃储存2 d后，仍能检测双酶级联到活性	[52]
聚合物囊泡(异氰肽与苯乙烯的嵌段共聚物)	GOx，HRP和CALB	—	[53]
聚合物囊泡[聚(2-甲基噁唑啉)-嵌段-聚(二甲基硅氧烷)-嵌段-聚(2-甲基噁唑啉)PMOXA-PDMS-PMOXA]	GOx，HRP和β-Gal	—	[54]
聚合物囊泡{聚苯乙烯-*b*-聚[3-(异氰基-丙氨酰-氨基乙基)噻吩](PS-*b*-PIAT)}	*N*-酰基-D-葡萄糖胺-2-烯丙基酶(AGE)，*N*-乙酰神经氨酸醛缩酶(NAL)和CMP-唾液酸合成酶(CSS)	—	[55]
聚合物囊泡{聚苯乙烯-*b*-聚[3-(异氰基-丙氨酰-氨基乙基)噻吩](PS-*b*-PIAT)}	PAMO，CALB和ADH	—	[56]
聚合物胶囊(天然多糖)	黄嘌呤氧化酶，尿酸酶和过氧化物酶	7个循环后产率是游离体系的2倍以上	[57]
聚合物胶囊(生物聚合物和碳酸钙)	β-葡萄糖苷酶(β-Glu)，GOx和HRP	在4 ℃保存1个月以上仍可以检测到活性	[58]
聚合物胶囊(藻酸盐和鱼精蛋白)	FDH和FalDH	可循环使用8次以上	[59]
聚合物胶囊(核酸功能化羧甲基纤维素水凝胶)	GOx和β-Gal	在4 ℃下保存3 d仍可检测到活性	[60]
聚合物胶囊[聚苯乙烯磺酸盐(PSS)和聚烯丙胺盐酸盐(PAH)]	人血清蛋白(HSA)	—	[61]
聚合物胶囊(聚烯丙胺盐酸盐和碳酸钙微粒)	*S*-3-羟丁酰辅酶A脱氢酶(DH)和黄素依赖型NADH氧化酶(NOx)	催化反应可进行72 h	[62]

白单体（α-溶血素），单体自发在囊泡的双分子层聚集形成蛋白孔（直径1.5 nm）。在蛋白孔的存在下，溶液中葡萄糖（分子直径1 nm）进入GOx区室，经GOx催化后产生H_2O_2。H_2O_2通过扩散作用进入HRP区室，在HRP的存在下，Amplex Red（10-乙酰基-3,7-二羟基吩嗪，过氧化物酶底物的荧光探针）与H_2O_2反应产生红色荧光物质。该实验证明了在聚合物囊泡中插入孔道蛋白能够运送底物进入反应区室，引发偶联反应。聚合物囊泡可以将小的囊泡包封在大的囊泡内部来模拟细胞器结构[32]。Peters等[56]制备了结构类似于真核细胞的多区室纳米反应器，南极假丝酵母CALB和ADH被分别封装在由聚苯乙烯-*b*-聚[3-(异氰基-丙氨酰-氨基乙基)噻吩]（PS-*b*-PIAT）形成的小型反应器中，再将多个小型反应器、苯丙酮单加氧酶（PAMO）以及其他底物试剂包封在由聚丁二烯-聚环氧乙烷（PB-*b*-PEO）形成的大聚合物囊泡中，形成“囊泡中囊泡”结构。利用苯丙酮单加氧酶、南极假丝酵母CALB以及ADH的连续催化，荧光产物间苯二酚产率达到了25%。这种“囊泡中囊泡”结构将不同的酶固定在连续的区室中，保证了每一步催化能够发生在特定的区室中，如图2所示。

尽管聚合物囊泡在仿生分区室固定化中已有研究，但仍存在一定的局限性。聚合物囊泡制备过程复杂烦琐，需要通过精心设计和复杂操作才能实现对聚合物囊泡结构、性质和功能的精准控制。此外，由于结构复杂，对酶分子的封装效率较低。

2.2 聚合物胶囊

聚合物胶囊[74-75]是由囊芯和聚合物胶囊膜组成的核壳纳米球体，其大小和形状均一，结构易调控，具有良好小分子的渗透性，可以封装并控制释放活性物质如蛋白质、多糖、多肽、核酸以及酶分子等，能够用于构建模拟细胞和细胞器的分区室微反应器[76-77]，如图3所示。其制备方法主要包括模板定向合成法[78]、层层自组装法[79]（Layer-by-Layer， LbL）、相分离法[80]、悬浮聚合

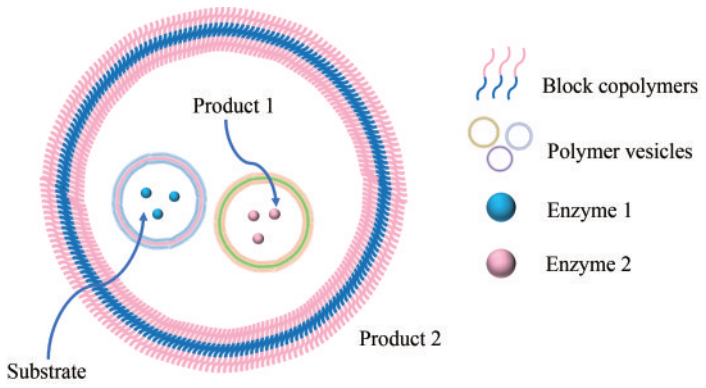


图2 聚合物囊泡分区室固定化多酶

Fig. 2 Immobilization of multi-enzyme by compartmentalized polymer vesicles

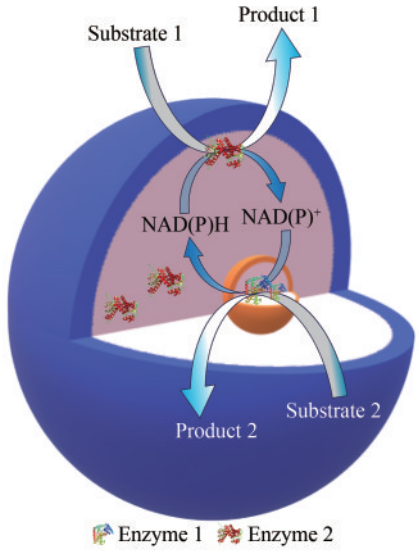


图3 聚合物胶囊分区室固定化多酶

Fig. 3 Immobilization of multi-enzyme by compartmentalized polymer capsules

法[81]、乳液聚合法[82]等。

聚合物胶囊优异的可回收性和形态可控性保证了其在复杂生物催化过程中的应用。Qu等[57]通过气体剪切法制备了包封黄嘌呤氧化酶（xanthine oxidase）、尿酸酶（uricase）和过氧化物酶（peroxidase）的囊芯微粒，接着通过表面凝胶化将海藻酸盐胶壳包覆在囊芯上制备多室微胶囊。黄嘌呤被黄嘌呤氧化酶催化生成尿酸和H_2O_2，接着由尿酸酶分解尿酸生成尿囊素、CO_2以及H_2O_2，过氧化物酶催化H_2O_2和底物邻苯二胺生成橙色产物2,3-二氨基吩嗪。该多室微胶囊由天然多糖组成，具有良好的生物相容性，且多室微胶囊的多酶偶联体系在7个循环之后的产率是游离酶级联体系的2倍以上。

聚合物胶囊在研究过程中也具有一定的局限性。聚合物胶囊易因反应溶剂、温度以及pH变化而发生变形，使得封装后的酶分子活性受到影响。此外，聚合物胶囊具有半透性，高分子量的底物难以通过胶囊进行扩散。聚合物的性质、胶囊外壳的厚度和密度、体系pH、温度以及离子强度都影响胶囊的物质传递性能[83]。

3 结论与展望

本文总结了MOF、聚合物囊泡以及聚合物胶囊在体外分区室固定化多酶偶联系统中的研究进展。将多种功能不同的酶固定在特定的区室中，形成适合酶催化的微环境提高反应效率和产物纯度[84-85]。MOF具有结构可控、表面易功能化以及合成简便等特点，是构建仿生分区室固定化多酶的良好材料；但在极端条件下（高温高压）结构易塌陷，并且采用共沉淀法固定化酶进行催化反应时，存在较大的传质阻力，限制反应速率。聚合物囊泡生物相容性好，尺寸分布范围广，有利于底物和产物传质，这些特点赋予了聚合物囊泡作为纳米反应器或仿生细胞器的可能性；但其制备过程复杂烦琐，酶包封率低。聚合物胶囊具有结构可控性好等优点；但成本较高，且受外界环境（温度、pH、溶剂）影响较大，结构和功能易发生改变，导致酶分子失活或泄漏。几种材料优缺点总结于表3。

在体外多酶协同催化体系中，多酶偶联合成

表3 材料优缺点

Table 3 The advantages and disadvantages of materials

材料	优点	缺点
MOF	性能可控性	极端条件下结构塌陷
	微观可控性	共沉淀固定化酶催化反应传质阻力大
聚合物囊泡	生物相容性好	酶包封率低
	尺寸分布范围广	制备过程烦琐复杂
聚合物胶囊	良好的可回收性	制备成本高
	形态可控	易受环境影响

通常伴随着分步反应不相容的问题[86]。一般情况下，多酶级联的效率很大程度上取决于参与偶联反应的分步反应速率[2]。因此在体外分区室固定化多酶体系中，需要找到多酶体系中的瓶颈酶。围绕瓶颈酶的酶活，根据各个酶的动力学参数，计算各个酶的配比，调节不同区室内的酶固载量，实现优化。同时通过调控分区室的微环境，尽量使各个酶处于最适反应条件，提升整体反应速率[87-88]。Chung等[89]开发了一种基于支链聚合物-硅壳酶微球（EMB）的体外模块化多酶级联系统，整个体系的反应速率和产率主要通过EMB数量进行调节。此外，将仿生分区室固定化技术的微观分区室与其他技术如微流控技术[90-91]宏观分区室相结合[92-93]，不同酶按照顺序固定在微流控装置中，通过流场用工程化的手段调控停留时间以及底物的不同区域浓度，也能解决体外多种酶的分步调控问题[94]，同时也丰富了仿生分区室固定化技术的应用场景如可穿戴设备[95]、生物检测传感器[96]等。

此外，体外多酶级联的稳定性是讨论载体材料用于固定化酶时必须要考虑的因素。缓冲溶液的组成和浓度、载体材料的结构和性质、固定化酶方式以及体系pH都对稳定性产生了影响[97-99]。Shortall等[100]制备了5种固定化酶MOF材料ALDHTt@Fe-BTC、ALDHTt@Co-TMA、ALDHTt@Ni-TMA、ALDHTt@Cu-TMA及ALDHTt@ZIF-zni，由于柠檬酸盐易与金属离子螯合，5种MOFs材料在柠檬酸溶液中易分解。棒状MOFs比纳米花状稳定性更差，ALDHTt@Co-TMA（11.4 μm±3.0 μm，棒状）和ALDHTt@Ni-TMA（7.9 μm±1.6 μm，棒状）相较于其他MOFs材料结构更易塌陷。

尽管仿生分区室多酶固定化技术已有较多的研究，但在工业生产中仍面对酶定向固定化、分区室模块接头规范化设计、易替换性以及降低生产成本等难题。未来，开发可精确调控、循环使用、高抗逆性的多酶分区室仿生反应体系是生物催化领域中的一项重要挑战。

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