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抗原性流感-铁蛋白多肽

(57) 摘要

本公开文本涉及抗原性流感-铁蛋白多肽及其在引发针对流感的抗体中的用途。

1. 一种抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽包含(i)含有用半胱氨酸置换表面暴露的氨基酸的突变的铁蛋白蛋白,和(ii)流感多肽。

2. 一种抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽包含(i)铁蛋白蛋白,所述铁蛋白蛋白含有用半胱氨酸置换表面暴露的氨基酸的突变和与所述半胱氨酸缀合的免疫刺激部分;和(ii)流感多肽。

3. 根据权利要求1所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含经由所述半胱氨酸缀合至所述铁蛋白蛋白的免疫刺激部分。

4. 根据权利要求1-3中任一项所述的抗原性流感-铁蛋白多肽,其中所述流感多肽包括血凝素(HA)或神经氨酸酶(NA)多肽。

5. 根据权利要求4所述的抗原性流感-铁蛋白多肽,其中所述HA多肽包含保守区。

6. 根据权利要求5所述的抗原性流感-铁蛋白多肽,其中所述保守区包含所述HA的茎区的全部或一部分。

7. 根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽,其中所述流感抗原包括含有Y98F突变的HA抗原。

8. 根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含用非半胱氨酸氨基酸置换内部半胱氨酸的突变。

9. 根据权利要求8所述的抗原性流感-铁蛋白多肽,其中所述内部半胱氨酸在幽门螺杆菌铁蛋白的位置31处,或如通过成对或结构比对确定的对应于幽门螺杆菌铁蛋白的位置31的位置处,任选地其中所述内部半胱氨酸突变成丝氨酸。

10. 根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含用非天冬酰胺氨基酸置换表面暴露的天冬酰胺的突变,任选地其中所述非天冬酰胺氨基酸是谷氨酰胺。

11. 根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽,其中所述表面暴露的氨基酸是幽门螺杆菌铁蛋白的E12、S26、S72、A75、K79、S100或S111或如通过成对或结构比对确定的非幽门螺杆菌铁蛋白中的类似氨基酸的突变。

12. 根据权利要求11所述的抗原性流感-铁蛋白多肽,其中所述表面暴露的氨基酸处的所述突变是幽门螺杆菌铁蛋白的E12C、S26C、S72C、A75C、K79C、S100C或S111C或如通过成对或结构比对确定的非幽门螺杆菌铁蛋白中的类似氨基酸。

13. 根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽,其中所述免疫刺激部分是TLR7或TLR8的激动剂。

14. 根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽,其中所述免疫刺激部分是TLR9的激动剂。

15. 根据权利要求1或3-14中任一项所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含在所述免疫刺激部分与所述铁蛋白蛋白之间的接头。

16. 根据权利要求15所述的抗原性流感-铁蛋白多肽,其中所述接头包括马来酰亚胺部分、聚乙二醇(PEG)部分和二苯并环辛炔(DBCO)部分中的一种、两种或三种。

17. 根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含在所述铁蛋白蛋白与所述流感多肽之间的肽接头。

18. 一种铁蛋白颗粒,所述铁蛋白颗粒包含根据前述权利要求中任一项所述的抗原性

流感-铁蛋白多肽。

19. 一种组合物,所述组合物包含根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽或铁蛋白颗粒和药学上可接受的载体。

20. 根据权利要求19所述的组合物,所述组合物进一步包含含有铁蛋白蛋白和不同的流感多肽的第二抗原性流感-铁蛋白多肽。

21. 根据权利要求20所述的组合物,其中所述流感多肽来自甲型流感并且所述第二抗原性流感-铁蛋白多肽的流感多肽来自乙型流感,或其中所述流感多肽和所述第二流感-铁蛋白多肽的流感多肽来自亚型H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15、H16、H17或H18,或其中所述流感多肽中的一种或两种包括来自亚型H1、H3、H7或H10的工程化的稳定化茎抗原。

22. 根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽、铁蛋白颗粒或组合物,用于在引发对流感的免疫应答或保护受试者免受流感感染的方法中使用。

23. 一种引发对流感的免疫应答或保护受试者免受流感感染的方法,所述方法包括向受试者给予根据前述权利要求中任一项所述的任何一种或多种抗原性流感-铁蛋白多肽、铁蛋白颗粒或组合物。

24. 根据前述权利要求中任一项所述的抗原性流感-铁蛋白多肽、铁蛋白颗粒、组合物或方法,其中所述受试者是人。

25. 一种核酸,所述核酸编码根据权利要求1-17中任一项所述的抗原性流感-铁蛋白多肽,任选地其中所述核酸是mRNA。

抗原性流感-铁蛋白多肽

[0001] 本申请要求2018年4月3日提交的美国临时专利申请号62/652,204的权益,所述临时专利申请的全部内容通过引用并入本文中。

[0002] 本申请含有已经以ASCII格式电子提交并且通过引用以其整体特此并入的序列表。2019年3月18日创建的所述ASCII副本被命名为2019-03-18_01121-0034-00PCT_SL.txt并且大小为285,731字节。

[0003] 即使在疫苗学领域取得了许多成功,仍需要新的突破来保护人类免受许多威胁生命的传染性疾病的影响。当前许多已获许可的疫苗都依赖十年前的技术来产生生活的减毒病原体或灭活的杀死病原体,所述病原体具有固有的安全性问题,并且在许多情况下,仅刺激短暂的弱免疫应答,从而需要给予多个剂量。虽然遗传学和生物化学工程学的进步使得开发针对具有挑战性的疾病靶标的治疗剂成为可能,但在疫苗学领域的这些应用尚未完全实现。重组蛋白技术现在允许设计最佳抗原。另外,纳米颗粒已越来越多地展示出用于最佳抗原呈递和靶向药物递送的潜力。已经显示具有多个附接抗原的纳米颗粒具有增加的结合亲合力,所述增加的结合亲合力凭借其分子货物的多价展示和由于其纳米尺寸而更有效地跨过生物屏障的能力而提供。与流感病毒血凝素(HA)蛋白融合的幽门螺杆菌(*Helicobacter pylori* (H.pylori))铁蛋白纳米颗粒可以改善抗原稳定性并增加在小鼠流感模型中的免疫原性(参见Kanekiyo等人,Nature 499:102-106 (2013))。此融合蛋白自组装成八面体对称性纳米颗粒并呈递8个三聚体HA刺突,从而在与佐剂一起使用时在各种临床前模型中产生稳健的免疫应答。

[0004] 流感仍然是全球严重的健康威胁,每年感染5%-10%的成年人并且每年造成250,000-500,000人死亡。由于生产时间长,疫苗株系必须在流感季节之前半年或更长时间被选出,并且经常因遗传漂移和漂变以及出现新株系而遭受失配。即使当正确匹配时,目前的疫苗也仅有50%-60%功效。因此,非常需要通过增加疫苗接种的广度、效力和/或耐久性来改善疫苗功效,并引入现代的生产实践。

[0005] 在许多季节中观察到的流感疫苗功效低的潜在实质因素在于,病毒倾向于通过易错复制而发生突变,这使得病毒可以逃避接种疫苗的个体中的中和抗体的结合。流感病毒上保守表位的存在提示了一种通向可以抵抗病毒漂变的广泛中和流感疫苗的途径。然而,为了引发针对这些保守表位的抗体,需要重组蛋白工程化以将这些表位以有利的方式呈递至免疫系统。例如,当将全长血凝素(HA)用作免疫原时,所引发的抗体主要是与据说呈免疫显性的高度可变头部结构域结合的那些抗体。已经设计出一种诱导针对次显性高度保守的茎区的抗体的策略,所述策略涉及“切断”HA的头部并通过蛋白质工程化捆绑剩余的蛋白质链(参见Yassine,Nature Medicine 21(9):1065-1071 (2015))。

[0006] 在此,呈现了在用于流感的疫苗治疗剂方面的一组新的进展。开发了抗原性流感-铁蛋白多肽,其中使工程化半胱氨酸置换铁蛋白中表面暴露的氨基酸。将免疫刺激部分(如佐剂)经由半胱氨酸缀合至抗原性多肽。由抗原性多肽组装的自佐剂化铁蛋白颗粒具有高度抗原性。将免疫刺激部分与铁蛋白颗粒的直接缀合与所述铁蛋白颗粒与流感多肽的融合相组合允许在单一大分子实体中靶向共递送免疫刺激部分和流感多肽,这可以大大降低全

身性毒性的可能性,所述全身性毒性是在包含作为单独分子的抗原和佐剂的更传统的疫苗的情况下所担忧的。此外,免疫刺激部分与流感多肽在大分子实体中的共递送以及它们的多价呈递也可以减少所需疫苗的总剂量,从而减少生产负担和成本。

发明内容

[0007] 本公开文本的目的是提供可以提供以上讨论的一个或多个优点,或者至少为公众提供有用选择的组合物、试剂盒、方法和用途。因此,本文公开了以下实施方案。

[0008] 实施方案1是一种抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽包含(i)含有用半胱氨酸置换表面暴露的氨基酸的突变的铁蛋白蛋白,和(ii)流感多肽。

[0009] 实施方案2是一种抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽包含(i)铁蛋白蛋白,所述铁蛋白蛋白含有用半胱氨酸置换表面暴露的氨基酸的突变和与所述半胱氨酸缀合的免疫刺激部分,和(ii)流感多肽。

[0010] 实施方案3是根据实施方案1所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含经由所述半胱氨酸缀合至所述铁蛋白蛋白的免疫刺激部分。

[0011] 实施方案4是根据实施方案1-3中任一项所述的抗原性流感-铁蛋白多肽,其中所述流感多肽包括血凝素(HA)或神经氨酸酶(NA)多肽。

[0012] 实施方案5是根据实施方案4所述的抗原性流感-铁蛋白多肽,其中所述HA多肽包含保守区。

[0013] 实施方案6是根据实施方案5所述的抗原性流感-铁蛋白多肽,其中所述保守区包含所述HA的茎区的全部或一部分。

[0014] 实施方案7是根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽,其中所述流感抗原包括含有Y98F突变的HA抗原。

[0015] 实施方案8是根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含用非半胱氨酸氨基酸置换内部半胱氨酸的突变。

[0016] 实施方案9是根据实施方案8所述的抗原性流感-铁蛋白多肽,其中所述内部半胱氨酸在幽门螺杆菌铁蛋白的位置31处,或如通过成对或结构比对确定的对应于幽门螺杆菌铁蛋白的位置31的位置处,任选地其中所述内部半胱氨酸突变成丝氨酸。

[0017] 实施方案10是根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含用非天冬酰胺氨基酸置换表面暴露的天冬酰胺的突变,任选地其中所述非天冬酰胺氨基酸是谷氨酰胺。

[0018] 实施方案11是根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽,其中所述表面暴露的氨基酸是幽门螺杆菌铁蛋白的E12、S26、S72、A75、K79、S100或S111或如通过成对或结构比对确定的非幽门螺杆菌铁蛋白中的类似氨基酸的突变。

[0019] 实施方案12是根据实施方案11所述的抗原性流感-铁蛋白多肽,其中所述表面暴露的氨基酸处的所述突变是幽门螺杆菌铁蛋白的E12C、S26C、S72C、A75C、K79C、S100C或S111C或如通过成对或结构比对确定的非幽门螺杆菌铁蛋白中的类似氨基酸。

[0020] 实施方案13是根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽,其中所述免疫刺激部分是TLR7或TLR8的激动剂。

[0021] 实施方案14是根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽,其中

所述免疫刺激部分是TLR9的激动剂。

[0022] 实施方案15是根据实施方案1或3-14中任一项所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含在所述免疫刺激部分与所述铁蛋白蛋白之间的接头。

[0023] 实施方案16是根据实施方案15所述的抗原性流感-铁蛋白多肽,其中所述接头包括马来酰亚胺部分、聚乙二醇(PEG)部分和二苯并环辛炔(DBCO)部分中的一种、两种或三种。

[0024] 实施方案17是根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽,所述抗原性流感-铁蛋白多肽进一步包含在所述铁蛋白蛋白与所述流感多肽之间的肽接头。

[0025] 实施方案18是一种铁蛋白颗粒,所述铁蛋白颗粒包含根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽。

[0026] 实施方案19是一种组合物,所述组合物包含根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽或铁蛋白颗粒和药学上可接受的载体。

[0027] 实施方案20是根据实施方案19所述的组合物,所述组合物进一步包含含有铁蛋白蛋白和不同的流感多肽的第二抗原性流感-铁蛋白多肽。

[0028] 实施方案21是根据实施方案20所述的组合物,其中所述流感多肽来自甲型流感并且所述第二抗原性流感-铁蛋白多肽的流感多肽来自乙型流感,或其中所述流感多肽和所述第二流感-铁蛋白多肽的流感多肽来自亚型H1、H2、H3、H4、H5、H6、H7、H8、H9、H10、H11、H12、H13、H14、H15、H16、H17或H18,或其中所述流感多肽中的一种或两种包括来自亚型H1、H3、H7或H10的工程化的稳定化茎抗原。

[0029] 实施方案22是根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽、铁蛋白颗粒或组合物,用于在引发对流感的免疫应答或保护受试者免受流感感染的方法中使用。

[0030] 实施方案23是一种引发对流感的免疫应答或保护受试者免受流感感染的方法,所述方法包括向受试者给予根据前述实施方案中任一项所述的任何一种或多种抗原性流感-铁蛋白多肽、铁蛋白颗粒或组合物。

[0031] 实施方案24是根据前述实施方案中任一项所述的抗原性流感-铁蛋白多肽、铁蛋白颗粒、组合物或方法,其中所述受试者是人。

[0032] 实施方案25是一种核酸,所述核酸编码根据实施方案1-17中任一项所述的抗原性流感-铁蛋白多肽,任选地其中所述核酸是mRNA。

[0033] 其他目的和优点将阐述于随后的描述中,并且/或者将从所述描述中明显看出,或者可以通过实践获知。所述目的和优点将借助所附权利要求中特别指出的要素和组合来实现和获得。

[0034] 应了解,上文的一般说明与下文的详细说明均仅是示例性和解释性的,并且对权利要求无限制性。

[0035] 并入本说明书并且构成本说明书的一部分的附图展示了某些实施方案,并且附图连同本说明一起用于解释本文所述的原理。

附图说明

[0036] 图1A至1B.代表性H1N1流感病毒株系的序列比较和同源性。(图1A)使用载体NTI AlignX软件对候选抗原性HA多肽的序列比对。白底黑字:源自给定位置处的完全保守残基的共有残基。黑底白字:源自给定位置处发生率大于50%的单一残基的共有残基。白底黑字加下划线和粗体:与给定位置处的共有残基弱相似的残基。白底黑字带有双下划线和斜体:源自给定位置处的相似残基块的共有残基。白底黑字加粗:非相似残基。示出的序列是以下序列的HA部分加上C末端处的接头丝氨酸:CA09 HA-Np=SEQ ID NO:15的残基1-519.COBRAP1 HA-Np=SEQ ID NO:27的残基1-519.COBRAX6 HA-Np=SEQ ID NO:29的残基1-518.NC99 HA-Np=SEQ ID NO:1的残基1-518.HK77 HA-Np=SEQ ID NO:18的残基1-519.FM47HA-Np=SEQ ID NO:17的残基1-519.DV57 HA-Np=SEQ ID NO:21的残基1-518.MAL54 HA-Np=SEQ ID NO:16的残基1-519。(图1B)用邻接法(Vector NTI)针对来自所列出流感株系的HA蛋白序列生成的系统树图。箭头指示通过从这些序列产生HA-铁蛋白纳米颗粒并在小鼠中测试其免疫原性而被选择作为用于评价的候选物的株系。

[0037] 图2.铁蛋白上的工程化的表面暴露的半胱氨酸(Cys)。在铁蛋白纳米颗粒的情境下示出了由置换表面暴露的氨基酸的突变产生的半胱氨酸的位置。

[0038] 图3A至图3B.To11样受体(TLR)激动剂与铁蛋白的缀合。(图3A)在铁蛋白纳米颗粒的情境下说明了将SM7/8a小分子经由含有马来酰亚胺反应性基团的PEG4接头与由置换铁蛋白中表面暴露的氨基酸的突变产生的半胱氨酸缀合的结果。(图3B)在铁蛋白纳米颗粒的情境下说明了通过马来酰亚胺-DBCO双官能接头和叠氮化物官能化的CpG试剂,使用2步式点击化学将CpG(SEQ ID NO:230)与由置换铁蛋白中表面暴露的氨基酸的突变产生的半胱氨酸缀合的结果。

[0039] 图4A至图4B.To11样受体(TLR)激动剂与铁蛋白的缀合。(图4A)包含HA多肽和3M-012的铁蛋白纳米颗粒的模型,所述3M-012经由马来酰亚胺-DBCO双官能接头和叠氮化物官能化的3M-012试剂,使用2步式点击化学反应而与铁蛋白的表面暴露的半胱氨酸缀合。(图4B)包含HA多肽和CpG(SEQ ID NO:231)的铁蛋白纳米颗粒的模型,所述CpG用马来酰亚胺-DBCO双官能接头和叠氮化物官能化的CpG试剂,使用2步式点击化学反应而与铁蛋白的表面暴露的半胱氨酸缀合。

[0040] 图5.某些铁蛋白纳米颗粒构建体的铁蛋白部分内的胰蛋白酶切割位点。一些铁蛋白纳米颗粒除了用于缀合佐剂的未配对表面暴露的半胱氨酸外还包含胰蛋白酶切割位点。图5中所呈现的氨基酸序列是“通用”序列,其示出了多个构建体共有的序列。例如,SEQ ID NO:14的残基519-694包含图5中所示的“通用”序列。“XXX”序列表示流感多肽。还指出了该序列中存在的铁蛋白S111C突变的位置。

[0041] 图6A和图6B.在伴有或不伴有缀合的情况下的凝胶移位和质谱(MS)结果。(图6A)H1/茎-Np(SEQ ID NO:43)在存在(+)或不存在(-)与马来酰亚胺-PEG4-SM7/8a的缀合的情况下的凝胶移位和质谱结果。(图6B)H5/hCobra2-Np(SEQ ID NO:32)在存在(+)或不存在(-)与马来酰亚胺-PEG4-SM7/8a的缀合的情况下的凝胶移位和质谱结果。

[0042] 图7A和图7B.在伴有或不伴有缀合的情况下的进一步凝胶移位结果。(图7A)在伴有或不伴有与马来酰亚胺-PEG4-SM7/8a或通过2步式点击化学与马来酰亚胺-PEG4-DBCO和叠氮化物-CpG的缀合的情况下,肽N-糖苷酶(PNGase)处理后的H1/茎-Np的凝胶移位结果。

(图7B)在伴有或不伴有与马来酰亚胺-PEG4-SM7/8a或通过2步式点击化学与马来酰亚胺-PEG4-DBCO和叠氮化物-CpG的缀合的情况下,胰蛋白酶处理后的H1/茎-Np的凝胶移位结果。

[0043] 图8A和图8B.在伴有或不伴有马来酰亚胺-PEG4-SM7/8a与H1/茎-Np的缀合的情况下的质谱。(图8A) PNGase处理后的MS数据显示出与马来酰亚胺-PEG4-SM7/8a缀合之前和之后的H1/茎-Np的质量。(图8B)用胰蛋白酶处理H1/茎-Np后的MS数据显示出与马来酰亚胺-PEG4-SM7/8a缀合之前和之后经切割铁蛋白的质量。

[0044] 图9.在伴有或不伴有马来酰亚胺-PEG4-DBCO接头与H1/茎-Np的缀合的情况下的质谱。通过在添加接头后由MS测量的质量变化来确认至H1/茎-Np的马来酰亚胺-PEG4-DBCO接头添加。

[0045] 图10.通过2步式点击化学缀合CpG之前和不同阶段的H1/茎-Np的SDS-PAGE。“叠氮化物-CpG之后”是指2步式点击反应的最终产物。

[0046] 图11.对包含3M-012的接头与NC99 HA-TEV-Np构建体的缀合的表征。WT和+TEV泳道中的构建体具有序列SEQ ID NO:13;S26C是指SEQ ID NO:10;S72C是指SEQ ID NO:11;A75C是指SEQ ID No:12;并且S111C是指SEQ ID NO:9。除WT外,所有样品均用烟草蚀纹病毒蛋白酶(TEV)处理。

[0047] 图12A至图12E.在伴有或不伴有还原或缀合的情况下各种构建体的质谱。(图12A)还原之前的包含S111C的H1/茎-Np。(图12B)还原之后的包含S111C的H1/茎-Np。还原之后观察到的质量下降(115Da)与去除抑制半胱氨酸反应性的翻译后修饰一致。(图12C)在伴有或不伴有与3M012的缀合的情况下NC99 HA-TEV-Np-S26C纳米颗粒(SEQ ID NO:10)的质谱。(图12D)在伴有或不伴有与3M012的缀合的情况下NC99 HA-TEV-Np-A75C纳米颗粒(SEQ ID NO:12)的质谱。(图12E)在伴有或不伴有与3M012的缀合的情况下NC99 HA-TEV-Np-S111C纳米颗粒(SEQ ID NO:9)的质谱。

[0048] 图13A至图13F.在伴有或不伴有与SM7/8a、3M012或CpG的缀合的情况下H1/茎-Np或NC99 HA-Np的纳米颗粒的负染色电子显微镜(EM)图像。(图13A)未缀合的H1/茎-Np。(图13B)H1/茎-Np-SM7/8a缀合物。(图13C)H1/茎-Np-CpG缀合物。(图13D)NC99 HA-Np(SEQ ID NO:9)。(图13E)NC99 HA-Np-3M012缀合物。(图13F).NC99 HA-Np-CpG缀合物。

[0049] 图14A至图14C.H1/茎-Np-SM7/8a缀合物(图14A)、H1/茎-Np-CpG缀合物(图14B)或未缀合的H1/茎-Np(图14C)的动态光散射(DLS)分析。

[0050] 图15A至图15B.对与掺合的如所指示佐剂一起配制或如图3A至图3B所示经由PEG4接头与TLR激动剂(如SM7/8a或CpG)缀合的H1/茎-Np的抗体应答。在免疫(第0周和第3周)后5周时从小鼠(n=5)收集血清,并且通过酶联免疫吸附测定(ELISA)测量抗体效价。该数据证明了H1/茎-Np-TLR-激动剂缀合物的自佐剂化特性。图15A示出了针对H1/新喀里多尼亚/20/1999HA三聚体的ELISA。PAA=聚丙烯酸;混合等摩尔=83.3ng SM7/8a(相当于用SM7/8缀合的铁蛋白纳米颗粒给予的剂量);高剂量=21.84μg(高于用SM7/8缀合的铁蛋白纳米颗粒给予的剂量)。图15B示出了针对H1/茎三聚体的ELISA。混合等摩尔=850ng CpG(相当于用CpG缀合的铁蛋白纳米颗粒给予的剂量);高剂量=20μg CpG。

[0051] 图16A至图16C.用伴有或不伴有缀合或分开的3M-012的NC99HA-Np(SEQ ID NO:9)获得的效价的比较。在小鼠中测试了对NC99HA-Np-3M012缀合的纳米颗粒(0.22μg/剂量)的抗体应答。掺合物对照包括HA-Np(0.22μg/剂量)和10μg 3M012(典型的文献剂量)的混合物

以及HA-Np (0.22 μ g/剂量) 和1.7ng 3M012(缀合物的等摩尔匹配物) 的混合物。另外的对照包括以匹配的HA含量 (0.17 μ g HA/剂量) 投配的未缀合HA-Np和IIV。基于ELISA终点效价(图16A)、假病毒(PsV) 中和IC50效价(图16B) 和血凝抑制(HAI) 效价(图16C), HA-Np-3M012缀合物诱导了比等摩尔掺合物对照显著更强的抗体应答。与未缀合的纳米颗粒和IIV护理标准相比, 缀合的纳米颗粒还诱导了更强的应答(ELISA) 和更强的中和抗体应答(PsV), 但是该结果在HAI分析中没有统计学显著性。测定是对于加强免疫后2周的血清的。一式三份地运行所有样品。对中值 $\pm$ SEM进行作图。**** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ 。

[0052] 图17A至图17C. 用伴有或不伴有缀合或分开的CpG的NC99HA-Np (SEQ ID NO:9) 获得的效价的比较。在小鼠中测试了对HA-Np-CpG缀合的纳米颗粒 (0.22 μ g/剂量) 的抗体应答。掺合物对照包括HA-Np (0.22 μ g/剂量) 和20 μ g CpG (典型的治疗性剂量) 的混合物以及HA-Np (0.22 μ g/剂量) 和21ng CpG (缀合物的等摩尔匹配物) 的混合物。另外的对照包括以匹配的HA含量 (0.17 μ g HA/剂量) 投配的未缀合HA-Np和IIV。基于ELISA终点效价(图17A) 和假病毒中和IC50效价(图17B), 与匹配的掺合物、未缀合的颗粒或IIV相比, 缀合物诱导更强的结合和中和抗体应答。HA-Np-CpG缀合物还诱导了比等摩尔掺合物对照显著更强的HAI效价(图17C)。另外, HAI效价比未缀合的HA-Ferr和IIV高2.6和3.0倍, 但是这些结果并不显著。ELISA和PsV测定是对于加强免疫后2周的血清的, 并且在加强免疫后5周对血清进行HAI分析。一式三份地运行所有样品, 并且对中值 $\pm$ SEM进行作图。**** $p < 0.0001$; *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ 。

[0053] 图18A至图18C. 对源自六个进化上相异的H1血凝素(HA) 抗原和两个计算生成的(COBRA) 抗原的自组装HA-铁蛋白纳米颗粒的表征。(图18A) 通过动态光散射(DLS) 测量纳米颗粒大小和多分散性(“分散性”)。(图18B) 通过SDS-PAGE考马斯染色评估HA纳米颗粒的纯度。(图18C) 通过负染色电子显微镜在80,000x放大倍率下使纳米颗粒完整性可视化。FM47=A/蒙茅斯堡/1-JY2/1947; MAL54=A/马来西亚/302/1954; DV57=A/丹佛/1957 (DV57); HK77=A/香港/117/1977; NC99=A/新喀里多尼亚/20/99; CA09=A/加利福尼亚/4/2009。COBRA P1和COBRA X6是从多个序列计算生成的共有序列, 最近由Carter DM等人, J Virol 90:4720-4734 (2016) 所述。对于SEQ ID NO参见图1A至图1B的图例。

[0054] 图19A至图19H. 由各种HA-铁蛋白纳米颗粒(对于SEQ ID NO参见图1A至图1B的图例) 引发的免疫应答的效力和广度。用所指示HA-Np疫苗免疫后6周, 针对一组相异的H1N1流感病毒, 对来自小鼠的血清的血凝抑制(HAI) 效价($\log_2$) 进行测定。在第0周和第3周, 用血凝素纳米颗粒(HA-Np) 对小鼠($n=5$) 进行免疫。虚线指示测定检测极限。(图19A至图19E) 伴有Ribi佐剂的数据。(图19F至图19H) 伴有AF03佐剂的数据。x轴线指示从1934年到2013年按参考年份测试的H1N1流感株系组。表2给出了对应于每年的完整株系名称。星号指示匹配的株系。

[0055] 图20A至图20F. 由HA-铁蛋白纳米颗粒诱导的HA抗体应答。(图20A) 用使用Sigma Adjuvant System(目录号S6322) 的指定纳米颗粒或纳米颗粒组合对小鼠 [$n=5$] 进行免疫。IIV是指流感灭活疫苗。(图20B至图20F) 通过确定对来自以下项的HA三聚体的ELISA效价来测量抗体应答: A/蒙茅斯堡/1/1947(图20B); A/马来西亚/302/1954(图20C); A/香港/117/1977(图20D); A/新喀里多尼亚/20/99(图20E); 或A/加利福尼亚/4/2009(图20F)。第一次免疫后5周测量ELISA效价。测试的最低血清稀释度设定了测定检测极限, 如通过虚线所指示

的。空心圆圈指示给予至小鼠的纳米颗粒的起源株系与通过ELISA测定的株系之间的匹配。

[0056] 图21A至图21H. 如通过HAI效价的测定所测量的, 对给予至小鼠的多种铁蛋白纳米颗粒的HA混合物的抗体应答。(图21A至图21B) 二价组合。(图21C至图21E) 三价组合。(图21F至图21H) 四价组合。测定了一组相异的H1N1流感病毒的HAI效价($\log_2$)。在伴有佐剂的情况下, 在第0周和第3周用所指示HA-纳米颗粒组合对小鼠($n=5$)进行免疫。在伴有AF03佐剂的情况下测试二价组合COBRA X6+COBRA P1 HA-Np, 以与单价COBRA HA-Np的评价相一致。同样, 单独株系HA-铁蛋白纳米颗粒的组合使用Ribi佐剂, 以与单价单独株系HA-Np的评价相一致。星号指示给予至小鼠的纳米颗粒的起源株系与通过ELISA测定的株系之间的匹配。x轴线指示从1934年到2013年按参考年份测试的H1N1流感株系组。表2给出了针对每年的完整株系名称。虚线指示测定检测极限。

[0057] 图22A至图22F. 如通过HAI效价的测定所测量的, 对伴有Ribi或AF03佐剂的组合物的抗体应答。对于NC99和CA09 HA-Np的二价组合(图22A至图22B), NC99、CA09和HK77 HA-Np的三价组合(图22C至图22D) 和NC99、CA09和FM47 HA-Np的三价组合(图22E至图22F), 用Ribi佐剂获得的结果(图22A、图22C和图22E) 相比于用AF03佐剂获得的结果(图22B、图22D和图22F) 是相似的。初免剂量后6周, 在小鼠血清中测定HAI效价, 其中在第3周进行加强免疫。在伴有Ribi或AF03佐剂的情况下, 用如所指示的HA-Np组合对小鼠[$n=5$]进行免疫。星号指示给予至小鼠的纳米颗粒的起源株系与通过ELISA测定的株系之间的匹配。x轴线指示从1934年到2013年按参考年份测试的H1N1流感株系组。表2给出了针对每年的完整株系名称。虚线指示测定检测极限。

[0058] 图23A至图23C. 如通过HAI效价的测定所测量的, 由蛋中产生的NC99和CA09灭活流感疫苗(IIV) 引发的比较性抗体应答。(图23A) NC99IIV。(图23B) CA09 IIV。(图23C) NC99+CA09 IIV。用所指示IIV疫苗(作为单一组分或共同给予的) 免疫后6周, 针对一组相异的H1N1流感病毒的来自小鼠的血清的血凝抑制(HAI) 效价($\log_2$)。在伴有Ribi佐剂的情况下, 在第0周和第3周用170ng (HA含量) 的每种疫苗对小鼠($n=5$) 进行免疫。x轴线指示从1934年到2013年按参考年份测试的H1N1流感株系组(也参见表2)。x轴线指示从1934年到2013年按参考年份测试的H1N1流感株系组。表2给出了针对每年的完整株系名称。虚线指示测定检测极限。

[0059] 图24A至图24DE. 如通过HAI效价的测定所测量的, 使用HA-铁蛋白纳米颗粒组合物或IIV对雪貂进行免疫后, 针对各种流感病毒的抗体应答。在使用掺合有AF03佐剂的以下项进行两次免疫后来自雪貂($n=12$ /组) 的血清的HAI效价($\log_2$): 图24A: 单独的PBS; 图24B: A/加利福尼亚/2009IIV; 图24C: NC99+CA09+HK77 HA-Np; 图24D: COBRA-X6+P1+HK77HA-Np。

[0060] 图24E. 对用HA-铁蛋白纳米颗粒组合物、IIV或PBS免疫的雪貂的异源攻击。如对于图24A至图24E所述那样对雪貂进行免疫, 并且在最后一次免疫后4周通过以下方式用1947蒙茅斯堡病毒攻击: 用1mL的A/蒙茅斯堡/1/1947病毒以50%组织培养物感染剂量(TCID₅₀)的 $10^{4.65}$ 倍进行鼻腔接种。在攻击后的七天时间历程中, 在鼻洗液中定量病毒效价。虚线指示测定检测极限。根据单尾不配对t检验和单向方差分析[F(3, 44)=5.18, $p=0.00375$], 与媒介物(PBS)对照相比, 在用HA-Np组合但未用CA09 IIV免疫的雪貂中, 病毒效价在攻击后第5天显著降低。 $***=p\leq 0.001$, 根据学生t检验。

[0061] 图25A至图25C. 用以下项免疫后的食蟹猴(长尾猕猴(*Macaca fascicularis*))的

抗体应答:50 μ g与掺合的AF03佐剂一起配制的H1/茎-Np、或200 μ g H1/茎-Np(无佐剂对照)、或200 μ g H1/茎-Np-SM7/8a缀合物(图3A中所示的)或200 μ g H1/茎-Np-CpG缀合物(图3B中所示的)。图25A示出了在所指示时间点(其中免疫在第0、4和10周进行)通过酶联免疫吸附测定(ELISA)在用H1/新喀里多尼亚/20/1999HA三聚体包被的板上测量的HA抗体效价。图25B示出了用H1/新喀里多尼亚/20/1999HA和NA假型化的慢病毒的中和IC₅₀。图25C示出了用H5/越南/1203/2004HA和NA假型化的慢病毒的中和IC₅₀。该数据证明了H1/茎-Np-TLR-激动剂缀合物在灵长类动物模型中的自佐剂化特性。

[0062] 图26A至图26B.用伴有或不伴有缀合或分开的3M-012的NC99HA-Np (SEQ ID NO:9)获得的效价的比较。对以下四种剂量的与NC99HA-Np-3M012缀合的纳米颗粒的抗体应答:0.1 μ g、0.5 μ g、2.5 μ g和12.5 μ g。包括了掺合物对照和未缀合的纳米颗粒用于比较。计算用于“等摩尔”掺合物对照和“高剂量”掺合物对照的TLR激动剂的量,以维持最初的缀合研究中使用的抗原与佐剂的比率(0.22 μ g HA-NP,对于“等摩尔”伴有1.7ng 3M012或对于“高剂量”伴有10 μ g)。在NC99慢病毒报告物测定(左,图26A)和NC99HAI测定(右,图26B)中测量血清中和。箭头突出显示了2.5 μ g剂量,其中HA-NP-3M012缀合物尽管含有低超过5000倍的3M012佐剂,但诱导了与HA-NP+3M012相似的抗体效价。对平均值 $\pm$ SEM进行作图。

具体实施方式

[0063] 本文提供用于针对流感免疫的抗原性流感-铁蛋白多肽。抗原性多肽包含流感多肽和铁蛋白,并且可以用于针对流感对受试者进行疫苗接种。铁蛋白允许与免疫刺激分子(如佐剂)缀合(例如,经由用半胱氨酸置换表面暴露的氨基酸的突变),从而消除了对单独给予的佐剂的需要,并且潜在地减少引发对流感多肽的免疫应答所需的总佐剂的量。还提供了包含抗原性流感-铁蛋白多肽的颗粒、组合物和试剂盒及其用途。还提供了编码本文所述多肽的核酸。

A. 定义

[0064] 如本文所用,“血凝素”或“HA”是指任何流感病毒的负责结合宿主细胞膜上的唾液酸的糖蛋白(示例性血凝素是UniProt登录号:P03451)。HA涵盖被抗HA抗体识别或可以引发抗HA抗体的合成多肽,如下文所述的COBRA P1、COBRA X6和COBRA X3。

[0065] 如本文所用,“HA茎”是指从缺乏完整HA头部的HA胞外结构域内的HA保守区设计的工程化流感多肽。关于保守的,其意指与作为整体的HA的序列同一性相比,所述区域在来自具有不同HA亚型的不同流感株系的HA之间保持显著更高的序列同一性。HA茎抗原例如详细讨论于以下文献中:Impagliazzo等人,Science 2015年9月18日,349(6254):1301-6; Valkenburg等人,Sci Rep.2016年3月7日,6:22666;Mallajosyula等人,Front Immunol.2015,6:329。

[0066] 如本文所用,“神经氨酸酶”或“NA”是指任何流感病毒的负责催化从病毒和细胞糖缀合物中去除末端唾液酸残基的糖蛋白(示例性神经氨酸酶为UniProt登录号:P03472)。

[0067] 如本文所用,“Y98F突变”是指用苯丙氨酸置换野生型HA序列中的与唾液酸直接接触的酪氨酸。由该突变产生的苯丙氨酸的位置显示在图1中。尽管在一些HA亚型中确切的位置可能有所不同,但可以通过序列比对或结构分析来鉴定。HA序列中存在Y98F突变意味着相应的野生型HA是包含与唾液酸直接接触的酪氨酸的亚型。

[0068] 如本文所用,“铁蛋白”或“铁蛋白蛋白”是指与幽门螺杆菌铁蛋白(SEQ ID NO:208或209)或本文所讨论的另一种铁蛋白(如激烈火球菌(*P.furiosus*)铁蛋白、粉纹夜蛾(*Trichoplusia ni*)铁蛋白或人铁蛋白)具有可检测的序列同一性的蛋白质,其用于例如在细胞内或组织中存储铁或在血流中携带铁。在下文详细讨论了此类示例性铁蛋白,包括以两条多肽链(称为重链和轻链)出现的那些(例如,粉纹夜蛾和人铁蛋白)。在一些实施方案中,铁蛋白包含与本文例如在表1(序列列表)中公开的铁蛋白序列具有至少15%、20%、30%、40%、50%、60%、70%、75%、80%、85%、90%、95%、97%、98%、99%或99.5%同一性的序列。铁蛋白可以是全长天然存在的序列的片段。

[0069] 如本文所用,“野生型铁蛋白”是指其序列由天然存在的序列组成的铁蛋白。铁蛋白还包括其氨基酸序列与野生型铁蛋白有一个或多个差异的全长铁蛋白或铁蛋白片段。

[0070] 如本文所用,“铁蛋白单体”是指未与其他铁蛋白分子组装的单一铁蛋白分子(或,在适用时,单一铁蛋白重链或轻链)。“铁蛋白多聚体”包含多个缔合的铁蛋白单体。“铁蛋白蛋白”包括单体铁蛋白和多聚体铁蛋白。

[0071] 如本文所用,“铁蛋白颗粒”是指已经自组装成球状形式的铁蛋白。铁蛋白颗粒有时被称为“铁蛋白纳米颗粒”或简称为“纳米颗粒”。在一些实施方案中,铁蛋白颗粒包含24个铁蛋白单体(或,在适用时,总共24个重链和轻链)。

[0072] 如本文所用,“杂合铁蛋白”是指包含幽门螺杆菌铁蛋白与牛蛙铁蛋白的氨基末端延伸区的铁蛋白。用作牛蛙铁蛋白的氨基末端延伸区的示例性序列呈现为SEQ ID NO:217。在杂合铁蛋白中,可以将牛蛙铁蛋白的氨基末端延伸区与幽门螺杆菌铁蛋白融合,由此使得使免疫刺激部分衔接位点均匀分布在铁蛋白颗粒表面。如本文所用,“牛蛙接头”是包含序列SEQ ID NO:217的接头。杂合铁蛋白有时也称为“bfpFerr”或“bfp铁蛋白”。可以在没有牛蛙序列的情况下,例如像在没有接头或在具有替代性接头的情况下提供包含牛蛙序列的任何构建体。示例性牛蛙接头序列提供于表1中。在表1示出牛蛙接头的情况下,可以在没有接头或具有替代性接头的情况下制造相同的构建体。

[0073] 如本文所用,“N-聚糖”是指在蛋白质的N(天冬酰胺)残基的酰胺氮处衔接至蛋白质的糖链。这样,通过N-糖基化过程形成N-聚糖。此聚糖可以是多糖。

[0074] 如本文所用,“糖基化”是指将糖单元添加至蛋白质。

[0075] 如本文所用,“免疫应答”是指免疫系统的细胞(如B细胞、T细胞、树突细胞、巨噬细胞或多形核细胞)对刺激物(如抗原或疫苗)的应答。免疫应答可以包括参与宿主防御应答的身体的任何细胞,例如包括分泌干扰素或细胞因子的上皮细胞。免疫应答包括但不限于先天性和/或适应性免疫应答。如本文所用,“保护性免疫应答”是指保护受试者免受感染(例如,预防感染或预防与感染相关的疾病的发生)的免疫应答。测量免疫应答的方法在本领域中是熟知的,并且包括例如通过测量淋巴细胞(如B或T细胞)的增殖和/或活性、细胞因子或趋化因子的分泌、炎症、抗体产生等。“抗体应答”是其中产生抗体的免疫应答。

[0076] 如本文所用,“抗原”是指引发免疫应答的因子;和/或当暴露或给予至生物体时由T细胞受体结合的因子(例如,当由MHC分子呈递时)或与抗体(例如,由B细胞产生)结合的因子。在一些实施方案中,抗原在生物体中引发体液应答(例如,包括产生抗原特异性抗体)。可替代地或另外,在一些实施方案中,抗原在生物体中引发细胞应答(例如,涉及其受体与抗原特异性相互作用的T细胞)。特定抗原可以在靶标生物体(例如,小鼠、兔、灵长类动物、

人)的一个或若干成员中引发免疫应答,但不能在靶标生物体物种的所有成员中引发免疫应答。在一些实施方案中,抗原在靶标生物体物种的至少约25%、30%、35%、40%、45%、50%、55%、60%、65%、70%、75%、80%、85%、90%、91%、92%、93%、94%、95%、96%、97%、98%、99%的成员中引发免疫应答。在一些实施方案中,抗原结合抗体和/或T细胞受体,并且在生物体中可能诱导或可能不诱导特定生理应答。在一些实施方案中,例如,抗原可以在体外结合抗体和/或T细胞受体,无论在体内是否发生这种相互作用。在一些实施方案中,抗原与特定体液或细胞免疫的产物(包括由异源免疫原诱导的那些)反应。抗原包括如本文所述的抗原性流感-铁蛋白多肽。

[0077] 如本文所用,“免疫刺激部分”是指共价附接至铁蛋白或抗原性铁蛋白多肽并可以激活免疫系统的组分(单独或当附接至铁蛋白或抗原性铁蛋白多肽时)的部分。示例性免疫刺激部分包括toll样受体(TLR)例如TLR4、7、8或9的激动剂。在一些实施方案中,免疫刺激部分是佐剂。

[0078] 如本文所用,“佐剂”是指非特异性增强对抗原的免疫应答的物质或媒介物。佐剂可以包括而限于其上吸附有抗原的矿物质(明矾、氢氧化铝或磷酸盐)的悬浮液;其中抗原溶液乳化在矿物油中或水中的油包水或水包油乳剂(例如,弗氏不完全佐剂)。有时包括杀死的分枝杆菌(例如,弗氏完全佐剂)以进一步增强抗原性。免疫刺激寡核苷酸(例如,CpG基序)也可用作佐剂(例如,参见美国专利号6,194,388;6,207,646;6,214,806;6,218,371;6,239,116;6,339,068;6,406,705;和6,429,199)。佐剂还可以包括生物分子,如Toll样受体(TLR)激动剂和共刺激分子。佐剂可以作为单独分子在组合物中给予或与抗原性流感-铁蛋白多肽共价结合(缀合)。

[0079] “抗原性流感-铁蛋白多肽”在本文中用于指包含铁蛋白和流感多肽的分子,其中所述分子具有关于流感多肽的抗原性。抗原性可以是作为较大构建体的一部分的流感多肽的特征。即,所述构建体可以充当产生针对流感多肽的抗体的抗原就足够了,而不管不伴有铁蛋白的流感多肽是否可以如此。在一些实施方案中,将流感多肽和铁蛋白在基因上融合为融合蛋白。在一些实施方案中,流感多肽和铁蛋白是非基因连接的,例如通过化学缀合。

[0080] 如本文所用,“自佐剂化”是指组合物或多肽包含铁蛋白和直接缀合至铁蛋白的免疫刺激部分,使得铁蛋白和免疫刺激部分在同一分子实体中。可以将抗原性流感-铁蛋白多肽与免疫刺激部分缀合以产生自佐剂化多肽。

[0081] 如本文所用,“表面暴露的”氨基酸是指蛋白质(例如,铁蛋白)中具有侧链的氨基酸残基,当所述蛋白质在多聚化后(如果适用)处于其天然的三维构象时,所述侧链可以被溶剂分子接触。因此,例如,在形成24聚体的铁蛋白的情况下,当铁蛋白组装为24聚体,例如组装为铁蛋白多聚体或铁蛋白颗粒时,表面暴露的氨基酸残基是其侧链可以被溶剂接触的氨基酸残基。

[0082] 如本文所用,“受试者”是指动物界的任何成员。在一些实施方案中,“受试者”是指人。在一些实施方案中,“受试者”是指非人动物。在一些实施方案中,受试者包括但不限于哺乳动物、鸟、爬行动物、两栖动物、鱼、昆虫和/或蠕虫。在某些实施方案中,非人受试者是哺乳动物(例如,啮齿动物、小鼠、大鼠、兔、猴、犬、猫、绵羊、牛、灵长类动物和/或猪)。在一些实施方案中,受试者可以是转基因动物、基因工程化动物和/或克隆。在本发明的某些实施方案中,受试者是成人、青少年或婴儿。在一些实施方案中,术语“个体”或“患者”被使用并

且旨在与“受试者”可互换。

[0083] 如本文所用,术语“疫苗接种(vaccination或vaccinate)”是指给予旨在例如对致病因子产生免疫应答的组合物。疫苗接种可以在暴露于致病因子和/或发生一种或多种症状之前、期间和/或之后给予,并且在一些实施方案中,在暴露于所述因子之前、期间和/或之后不久给予。在一些实施方案中,疫苗接种包括间隔适当时间的疫苗接种组合物的多次给予。

[0084] 本公开文本描述了分别与给定核酸序列或氨基酸序列(参考序列)具有一定同一性程度的核酸序列和氨基酸序列。

[0085] 两个核酸序列之间的“序列同一性”指示序列之间相同的核苷酸的百分比。两个氨基酸序列之间的“序列同一性”指示序列之间相同的氨基酸的百分比。

[0086] 术语“相同%”、“同一性%”或类似术语旨在具体是指在待比较的序列之间的最佳比对中相同的核苷酸或氨基酸的百分比。所述百分比是纯粹统计学的,并且两个序列之间的差异可以但不一定随机分布在待比较的序列的整个长度上。两个序列的比较通常通过以下方式进行:在最佳比对之后,关于区段或“比较窗口”比较所述序列,以鉴定相应序列的局部区域。用于比较的最佳比对可以手动地进行或借助于Smith和Waterman,1981,AdS App.Math.2,482的局部同源性算法、借助于Needleman和Wunsch,1970,J.Mol.Biol.48,443的局部同源性算法、借助于Pearson和Lipman,1988,Proc.Natl Acad.Sci.USA 88,2444的相似性搜索算法、或借助于使用所述算法的计算机程序(在Wisconsin序列分析包(Genetics Computer Group,575Science Drive,威斯康星州麦迪逊)中的GAP、BESTFIT、FASTA、BLAST P、BLASTN和TFASTA)进行。

[0087] 百分比同一性通过以下方式获得:确定待比较的序列对应的相同位置的数目,用此数目除以比较的位置的数目(例如,参考序列中的位置的数目),并且然后将此结果乘以100。

[0088] 在一些实施方案中,同一性程度是针对区域给出的,所述区域是参考序列的整个长度的至少约50%、至少约60%、至少约70%、至少约80%、至少约90%或约100%。例如,如果参考核酸序列由200个核苷酸组成,则针对至少约100、至少约120、至少约140、至少约160、至少约180或约200个核苷酸(在一些实施方案中为连续核苷酸)给出同一性程度。在一些实施方案中,针对参考序列的整个长度给出同一性程度。

[0089] 分别与给定核酸序列或氨基酸序列具有特定同一性程度的核酸序列或氨基酸序列可具有所述给定序列的至少一种功能特性,例如并且在一些情况下,在功能上等同于所述给定序列。一个重要的特性包括充当细胞因子的能力,特别是当给予受试者时。在一些实施方案中,与给定核酸序列或氨基酸序列具有一定同一性程度的核酸序列和氨基酸序列在功能上等同于所述给定序列。

[0090] 如本文所用,术语“试剂盒”是指一组经包装的相关组分,如一种或多种化合物或组合物和一种或多种相关材料,如溶剂、溶液、缓冲液、说明书或干燥剂。

B. 抗原性流感-铁蛋白多肽

[0091] 本文提供了抗原性多肽,所述抗原性多肽包含流感多肽和铁蛋白,所述铁蛋白包含用半胱氨酸置换表面暴露的氨基酸的突变。多肽在与作为单独分子的佐剂一起给予和/或作为可以自佐剂化的纳米颗粒(例如,铁蛋白颗粒)的一部分给予时可以是抗原性的。

1. HA和NA多肽

[0092] 在一些实施方案中,流感多肽是包含全长或部分长度的HA或NA的HA或NA多肽。可以使用任何HA或NA多肽。HA或NA多肽可以是天然存在的或从天然改变的。在一些实施方案中,HA来自H1-H18中的任一种。在一些实施方案中,NA来自N1-N11中的任一种。

[0093] 在一些实施方案中,HA多肽包含HA胞外结构域。HA胞外结构域可能来自任何流感亚型,包括H1-H18。

[0094] 在一些实施方案中,HA多肽包含HA的茎区。HA的茎区可以来自任何流感亚型,包括H1-H18。

[0095] 在一些实施方案中,HA多肽来自甲型流感病毒。甲型流感病毒可以是A/波多黎各/1934、A/Weiss/1/1943、A/蒙茅斯堡/1/1947 (FM47)、A/马来西亚/302/54 (MAL54)、A/丹佛/1/1957 (DV57)、A/新泽西州/8/1976、A/USSR/90/1977、A/香港/117/1977 (HK77)、A/巴西/11/1978、A/智利/1/1983、A/台湾/1/1986、A/德克萨斯州/36/1991、A/北京/262/1995、A/新喀里多尼亚/20/1999 (NC99)、A/所罗门群岛/6/2006、A/布里斯班/59/2007、A/加利福尼亚/07/2009 (CA09)、A/孟加拉国/2021/2012或A/越南/3050/2013。

[0096] 在一些实施方案中,HA多肽来自H1流感病毒。在一些实施方案中,H1病毒是A/南卡罗来纳州/1/18。

[0097] 在一些实施方案中,HA多肽来自H2流感病毒。在一些实施方案中,H2病毒是1957大流行性H2N2甲型流感病毒。

[0098] 在一些实施方案中,HA多肽来自H3流感病毒。在一些实施方案中,H3流感病毒是H3N8病毒。在一些实施方案中,H3N8病毒是马俄亥俄州2003。在一些实施方案中,H3N8病毒是马巴里2005。在一些实施方案中,H3N8病毒是马阿博因2003。在一些实施方案中,H3流感病毒是H3N2病毒。在一些实施方案中,H3N2病毒是伯斯2009。在一些实施方案中,H3N2病毒是维多利亚2011。

[0099] 在一些实施方案中,HA多肽来自H5流感病毒。在一些实施方案中,H5流感病毒是H5/N1病毒。在一些实施方案中,H5/N1病毒是印度尼西亚2005。在一些实施方案中,H5/N1病毒是斑头雁2005。在一些实施方案中,H5/N1病毒是大天鹅2005。在一些实施方案中,H5/N1病毒是野鸭/华东2003。

[0100] 在一些实施方案中,HA多肽来自乙型流感病毒。在一些实施方案中,乙型病毒是威斯康星州2010。在一些实施方案中,乙型病毒是马萨诸塞州2012。在一些实施方案中,乙型病毒是普吉岛2013。在一些实施方案中,乙型病毒是布里斯班2008。在一些实施方案中,布里斯班2008序列包含D197N突变。发现此突变改善了此纳米颗粒的表达,并且在其他株系(如B/布里斯班/2009和B/普吉岛/2013)中是天然存在的突变。此氨基酸可参与接触唾液酸受体。

[0101] 在一些实施方案中,HA多肽包含根据Giles BM和Ross TM Vaccine 29(16):3043-54(2011)或Carter DM等人,J Virol 90:4720-4734(2016)的实施例产生的计算优化的广泛反应性抗原(COBRA)。

[0102] 在一些实施方案中,从人H1N1流感序列产生COBRA序列。在一些实施方案中,从跨越1999-2012的人H1N1流感序列产生COBRA序列。从跨越1999-2012的人H1N1流感序列产生的示例性COBRA序列是包含在SEQ ID NO:29中的COBRA X6。在一些实施方案中,从跨越

1933-1957年和2009-2011年的人H1N1株系加上来自1931-1998年的猪H1N1流感株系产生COBRA序列。从跨越1933-1957年和2009-2011年的人H1N1株系加上来自1931-1998年的猪H1N1流感株系产生的示例性COBRA序列是包含在SEQ ID NO:27中的COBRA P1。

[0103] 在一些实施方案中,COBRA序列是X3。在一些实施方案中,COBRA序列是从H5N1产生的hCOBRA-2。

[0104] 消除HA受体结合位点的突变(Y98F)在Whittle等人,Journal of Virology 11(8):4047-4057(2014)中进行了描述。在一些实施方案中,HA多肽包含Y98F突变。上文提出的任何HA可以被修饰为包含Y98F突变。在一些实施方案中,HA来自H1/新喀里多尼亚/1999(NC99)病毒,并且包含Y98F突变。

2. 铁蛋白

[0105] 铁蛋白蛋白自组装成包含多个单独单体的球状蛋白复合物。自组装的铁蛋白复合物可以称为铁蛋白颗粒或纳米颗粒。

[0106] 铁蛋白基因存在于许多物种中,并且尽管有序列变异,但通常显示出高度保守的 α -螺旋结构。这样,任何铁蛋白都可以用于本发明,包括细菌、昆虫和人铁蛋白,但是其与任何特别描述的铁蛋白具有序列同一性。

[0107] 在一些实施方案中,铁蛋白是细菌、昆虫、真菌、鸟类或哺乳动物的。在一些实施方案中,铁蛋白是人的。在一些实施方案中,铁蛋白是细菌的。在一些实施方案中,铁蛋白是幽门螺杆菌铁蛋白。

[0108] 在一些实施方案中,铁蛋白是轻链和/或重链铁蛋白。在一些实施方案中,铁蛋白是任选地具有本文所述的一种或多种修饰的人重链铁蛋白(FTH1, GENE ID No:2495)或人轻链铁蛋白(FTL, GENE ID No:2512)。在一些实施方案中,铁蛋白是任选地具有本文所述的一个或多个突变的粉纹夜蛾重链铁蛋白(GenBank:AY970291.1)或粉纹夜蛾轻链铁蛋白(AY970292.1)。在一些实施方案中,铁蛋白纳米颗粒包含重链铁蛋白和轻链铁蛋白的24个总亚基,例如12个重链亚基和12个轻链亚基。在一些实施方案中,铁蛋白包含用半胱氨酸置换表面暴露的氨基酸的突变。

[0109] 在一些实施方案中,提供了包含流感多肽的抗原性流感-铁蛋白多肽,所述流感多肽的长度足以使所述分子具有关于流感多肽的抗原性。

[0110] 在一些实施方案中,抗原性铁蛋白多肽包含轻链铁蛋白和流感多肽。在一些实施方案中,抗原性铁蛋白多肽包含重链铁蛋白和流感多肽。在一些实施方案中,可以将轻链铁蛋白和流感多肽与不具有流感多肽的重链铁蛋白组装。在一些实施方案中,可以将重链铁蛋白和流感多肽与不具有流感多肽的轻链铁蛋白组装。不具有非铁蛋白多肽(例如,流感多肽)的铁蛋白可以称为“裸铁蛋白”。

[0111] 在一些实施方案中,抗原性流感-铁蛋白多肽包含重链铁蛋白和流感多肽,或轻链铁蛋白和流感多肽。可以组合此类多肽以允许在单一铁蛋白多聚体或颗粒上表达两种相同或不同的流感多肽。在一些实施方案中,两种不同的流感多肽从两种不同的感染原(例如,不同株系或类型的流感)获得,并且附接至用于组装成纳米颗粒的重链和轻链铁蛋白。

[0112] 在一些实施方案中,抗原性铁蛋白多肽包含与轻链铁蛋白和第二流感多肽一起组装的重链铁蛋白和第一流感多肽以产生二价疫苗。在一些实施方案中,铁蛋白是具有本文所述的一个或多个突变的幽门螺杆菌铁蛋白(对于示例性幽门螺杆菌铁蛋白序列参见SEQ

ID NO:208或209)。在一些实施方案中,幽门螺杆菌铁蛋白(或其他细菌铁蛋白)与人铁蛋白之间较低的序列同源性在用作疫苗平台时可降低自身免疫的潜力(参见Kanekiyo等人,Cell 162,1090-1100(2015))。

[0113] 在一些实施方案中,铁蛋白是具有本文所述的一个或多个突变的激烈火球菌铁蛋白(NCBI seq WP_011011871.1)。

[0114] 在一些实施方案中,铁蛋白包含与野生型铁蛋白具有大于70%、大于75%、大于80%、大于85%、大于90%、大于95%、大于97%、大于98%、或大于99%同一性的序列。

a) 铁蛋白突变

[0115] 在一些实施方案中,本文公开了包含一个或多个突变的铁蛋白。在一些实施方案中,一个或多个突变包括野生型铁蛋白的氨基酸序列的改变和/或例如在N或C末端的插入。在一些实施方案中,与野生型铁蛋白相比,(在一些实施方案中,除了任何N末端插入),铁蛋白中的一个、两个、三个、四个、五个或更多个不同的氨基酸被突变。一个或多个突变可以改变铁蛋白的功能特性,例如,如下文详细讨论的。通常,突变简单地是指相对于相应的野生型铁蛋白的序列差异(如取代、添加或缺失的一个或多个氨基酸残基)。

(1) 用于缀合的半胱氨酸

[0116] 在一些实施方案中,铁蛋白被突变以提供用于缀合免疫刺激部分和/或流感多肽的化学处理。这可以通过用半胱氨酸置换表面暴露的非半胱氨酸氨基酸的突变来实现。为了避免疑问,如“用半胱氨酸置换表面暴露的氨基酸”的语言必定意味着野生型或突变前序列中的表面暴露的氨基酸不是半胱氨酸。提供用于缀合免疫刺激部分或流感多肽的化学处理的另一种方法是包括在铁蛋白的N或C末端的氨基酸区段(如接头),其中所述氨基酸区段包含半胱氨酸。在一些实施方案中,此半胱氨酸(无论是置换表面暴露的氨基酸还是在N或C末端接头中)是不成对的,这意指它没有合适的伴侣半胱氨酸来形成二硫键。在一些实施方案中,此半胱氨酸不改变铁蛋白的二级结构。在一些实施方案中,此半胱氨酸不改变铁蛋白的三级结构。

[0117] 在一些实施方案中,此半胱氨酸可以用于将药剂(如免疫刺激部分)与铁蛋白缀合。在一些实施方案中,此半胱氨酸提供了反应性的游离硫醇基团。在一些实施方案中,与铁蛋白上的此半胱氨酸缀合的药剂暴露在组装的铁蛋白颗粒的表面上。在一些实施方案中,在组装铁蛋白颗粒的同时,此半胱氨酸可以在给予后与受试者的分子和细胞相互作用。

[0118] 在一些实施方案中,此半胱氨酸的存在允许缀合一个或多个免疫刺激部分,例如佐剂。在一些实施方案中,在不存在此半胱氨酸的情况下不会发生免疫刺激部分的缀合。

[0119] 在一些实施方案中,被半胱氨酸置换的非半胱氨酸氨基酸选自幽门螺杆菌铁蛋白的E12、S72、A75、K79、S100和S111。因此,在一些实施方案中,被置换为半胱氨酸的表面暴露的氨基酸是对应于幽门螺杆菌铁蛋白的E12、S26、S72、A75、K79、S100或S111的氨基酸残基。通过成对或结构比对可以在非幽门螺杆菌铁蛋白中找到类似的氨基酸。在一些实施方案中,被半胱氨酸置换的非半胱氨酸氨基酸可以选自对应于人轻链铁蛋白的S3、S19、S33、I82、A86、A102和A120的氨基酸。在一些实施方案中,基于以下理解来选择要被半胱氨酸置换的表面暴露的氨基酸:如果天然氨基酸被半胱氨酸置换,它将在组装的铁蛋白多聚体或颗粒中是反应性的和/或此半胱氨酸不会破坏铁蛋白多聚体或颗粒的稳定性和/或此半胱氨酸不会导致铁蛋白表达水平降低。

[0120] 在一些实施方案中,铁蛋白包含E12C突变。在一些实施方案中,E12C残基可以用于将药剂(例如,免疫刺激部分和/或流感多肽)与铁蛋白缀合。在一些实施方案中,E12C残基提供了反应性的游离硫醇基团。在一些实施方案中,与铁蛋白单体上的E12C残基缀合的药剂在组装的铁蛋白多聚体或颗粒的表面上表达。在一些实施方案中,铁蛋白多聚体或颗粒的表面上存在二十四个E12C残基(每个单体中一个)。

[0121] 在一些实施方案中,铁蛋白包含S26C突变。在一些实施方案中,S26C残基可以用于将药剂(例如,免疫刺激部分和/或流感多肽)与铁蛋白缀合。在一些实施方案中,S26C残基提供了反应性的游离硫醇基团。在一些实施方案中,与铁蛋白单体上的S26C残基缀合的药剂在组装的铁蛋白多聚体或颗粒的表面上表达。在一些实施方案中,铁蛋白多聚体或颗粒的表面上存在二十四个S26C残基(每个单体中一个)。

[0122] 在一些实施方案中,铁蛋白包含S72C突变。在一些实施方案中,S72C残基可以用于将药剂(例如,免疫刺激部分和/或流感多肽)与铁蛋白缀合。在一些实施方案中,S72C残基提供了反应性的游离硫醇基团。在一些实施方案中,与铁蛋白单体上的S72C残基缀合的药剂在组装的铁蛋白多聚体或颗粒的表面上表达。在一些实施方案中,铁蛋白多聚体或颗粒的表面上存在二十四个S72C残基(每个单体中一个)。

[0123] 在一些实施方案中,铁蛋白包含A75C突变。在一些实施方案中,A75C残基可以用于将药剂(例如,免疫刺激部分和/或流感多肽)与铁蛋白缀合。在一些实施方案中,A75C残基提供了反应性的游离硫醇基团。在一些实施方案中,与铁蛋白单体上的A75C残基缀合的药剂在组装的铁蛋白多聚体或颗粒的表面上表达。在一些实施方案中,铁蛋白多聚体或颗粒的表面上存在二十四个A75C残基(每个单体中一个)。

[0124] 在一些实施方案中,铁蛋白包含K79C突变。在一些实施方案中,K79C残基可以用于将药剂(例如,免疫刺激部分和/或流感多肽)与铁蛋白缀合。在一些实施方案中,K79C残基提供了反应性的游离硫醇基团。在一些实施方案中,与铁蛋白单体上的K79C残基缀合的药剂在组装的铁蛋白多聚体或颗粒的表面上表达。在一些实施方案中,铁蛋白多聚体或颗粒的表面上存在二十四个K79C残基(每个单体中一个)。

[0125] 在一些实施方案中,铁蛋白包含S100C突变。在一些实施方案中,S100C残基可以用于将药剂(例如,免疫刺激部分和/或流感多肽)与铁蛋白缀合。在一些实施方案中,S100C残基提供了反应性的游离硫醇基团。在一些实施方案中,与铁蛋白单体上的S100C残基缀合的药剂在组装的铁蛋白多聚体或颗粒的表面上表达。在一些实施方案中,铁蛋白多聚体或颗粒的表面上存在二十四个S100C残基(每个单体中一个)。

[0126] 在一些实施方案中,铁蛋白包含S111C突变。在一些实施方案中,S111C残基可以用于将药剂(例如,免疫刺激部分和/或流感多肽)与铁蛋白缀合。在一些实施方案中,S111C残基提供了反应性的游离硫醇基团。在一些实施方案中,与铁蛋白单体上的S111C残基缀合的药剂在组装的铁蛋白多聚体或颗粒的表面上表达。在一些实施方案中,铁蛋白多聚体或颗粒的表面上存在二十四个S111C残基(每个单体中一个)。

(2) 内部半胱氨酸的去除

[0127] 在一些实施方案中,铁蛋白包含用非半胱氨酸氨基酸置换内部半胱氨酸的突变。去除天然内部半胱氨酸残基可以确保每个铁蛋白单体只有一个未配对的半胱氨酸,并且避免了不希望的反应(如二硫化物形成),并且可以导致更稳定且高效的结果(例如,佐剂呈

递)。在一些实施方案中,幽门螺杆菌铁蛋白的C31被非半胱氨酸氨基酸置换。在一些实施方案中,用丝氨酸(C31S)置换幽门螺杆菌铁蛋白的C31,但是可以使用任何非半胱氨酸残基,例如丙氨酸、甘氨酸、苏氨酸或天冬酰胺。通过成对或结构比对可以在非幽门螺杆菌铁蛋白中找到类似的氨基酸。因此,在一些实施方案中,被置换为非半胱氨酸的内部半胱氨酸是与幽门螺杆菌铁蛋白的C31对齐的氨基酸残基。显示C31S突变的示例性铁蛋白序列在SEQ ID NO:201-207中示出。在一些实施方案中,当铁蛋白中存在多于一个内部半胱氨酸时,两个或更多个(例如,每个)内部半胱氨酸被非半胱氨酸氨基酸置换,所述非半胱氨酸氨基酸如丝氨酸或选自丝氨酸、丙氨酸、甘氨酸、苏氨酸或天冬酰胺的氨基酸。

(3) 糖基化

[0128] 人相容性糖基化可有助于重组药物产品的安全性和功效。监管批准可能取决于证明适当的糖基化是关键的质量属性(参见Zhang等人,Drug Discovery Today 21(5):740-765(2016))。N-聚糖可以由天冬酰胺侧链的糖基化产生,并且在人与其他生物体(如细菌和酵母)之间的结构可能不同。因此,可能期望减少或消除根据本公开文本的铁蛋白中的非人糖基化和/或N-聚糖形成。在一些实施方案中,控制铁蛋白的糖基化改善了组合物的功效和/或安全性,尤其是当用于人疫苗接种时。

[0129] 在一些实施方案中,铁蛋白被突变以抑制N-聚糖的形成。在一些实施方案中,与其相应的野生型铁蛋白相比,突变的铁蛋白具有降低的糖基化。

[0130] 在一些实施方案中,铁蛋白包含用非天冬酰胺氨基酸置换表面暴露的天冬酰胺的突变。在一些实施方案中,表面暴露的天冬酰胺是幽门螺杆菌铁蛋白的N19或如通过成对或结构比对确定的对应于幽门螺杆菌铁蛋白的位置31的位置。在一些实施方案中,使这种天冬酰胺(例如幽门螺杆菌铁蛋白的N19)突变降低了铁蛋白的糖基化。在一些实施方案中,突变是用谷氨酰胺置换了天冬酰胺。在一些实施方案中,铁蛋白是包含N19Q突变的幽门螺杆菌铁蛋白。SEQ ID NO:201-207是包含N19Q突变的示例性铁蛋白序列。

[0131] 暴露于细菌或酵母中产生的糖基化蛋白的哺乳动物可能会对糖基化蛋白产生免疫应答,因为细菌或酵母中给定蛋白的糖基化模式可能与哺乳动物中相同蛋白的糖基化模式不同。因此,一些糖基化的治疗性蛋白质可能不适合在细菌或酵母中生产。

[0132] 在一些实施方案中,通过氨基酸突变进行的铁蛋白糖基化减少有利于蛋白质在细菌或酵母中的产生。在一些实施方案中,减少的铁蛋白糖基化降低了在给予在细菌或酵母中表达的突变铁蛋白后在哺乳动物中产生不利影响的可能性。在一些实施方案中,因为糖基化减少,所以在细菌或酵母中产生的突变铁蛋白在人受试者中的反应原性降低。在一些实施方案中,与野生型铁蛋白相比,用具有减少的糖基化的突变铁蛋白治疗后,人受试者中超敏反应的发生率较低。

[0133] 在一些实施方案中,与包含野生型铁蛋白的组合物或包含具有野生型糖基化的相应铁蛋白的组合物相比,包含具有减少的糖基化的突变铁蛋白的组合物在受试者中的降解较慢。在一些实施方案中,与包含野生型铁蛋白的组合物或包含具有野生型糖基化的相应铁蛋白的组合物相比,包含具有减少的糖基化的突变铁蛋白的组合物在受试者中具有降低的清除率。在一些实施方案中,与野生型铁蛋白或包含具有野生型糖基化的相应铁蛋白的组合物相比,包含具有减少的糖基化的突变铁蛋白的组合物具有更长的血清半衰期。

(4) 突变的组合

[0134] 在一些实施方案中,铁蛋白包含本文所述的多于一种类型的突变。在一些实施方案中,铁蛋白包含一种或多种独立地选自以下项的突变:减少糖基化的突变、去除内部半胱氨酸的突变和产生表面暴露的半胱氨酸的突变。在一些实施方案中,铁蛋白包含减少糖基化的突变、去除内部半胱氨酸的突变和产生表面暴露的半胱氨酸的突变。

[0135] 在一些实施方案中,铁蛋白包含N19Q突变、C31S突变和产生表面暴露的半胱氨酸的突变。在一些实施方案中,铁蛋白包含N19Q突变、C31S突变和E12C突变。在一些实施方案中,铁蛋白包含N19Q突变、C31S突变和S72C突变。在一些实施方案中,铁蛋白包含N19Q突变、C31S突变和A75C突变。在一些实施方案中,铁蛋白包含N19Q突变、C31S突变和K79C突变。在一些实施方案中,铁蛋白包含N19Q突变、C31S突变和S100C突变。在一些实施方案中,铁蛋白包含N19Q突变、C31S突变和S111C突变。在一些实施方案中,铁蛋白包含对应于前述突变组中的任一种的突变,其中相应的突变在通过铁蛋白氨基酸序列与幽门螺杆菌铁蛋白氨基酸序列 (SEQ ID NO:208或209) 的成对比对确定的位置处将N改变为Q、将C改变为S,并且将非半胱氨酸表面暴露的氨基酸改变为半胱氨酸。

[0136] 包含多于一种类型的突变的示例性铁蛋白提供于SEQ ID NO:201-207中。

3. 接头

[0137] 流感多肽和铁蛋白可以经由接头连接以提供抗原性流感铁蛋白多肽。在一些实施方案中,接头将流感多肽的氨基酸序列与铁蛋白的氨基酸序列分开。可以使用任何接头。在一些实施方案中,铁蛋白经由肽接头连接至HA多肽。这可以有利于抗原性铁蛋白多肽作为融合蛋白的表达(例如,从单一开放阅读框)。在一些实施方案中,接头是甘氨酸-丝氨酸接头。在一些实施方案中,甘氨酸-丝氨酸接头是GS、GGGS (SEQ ID NO:226)、2XGGGS (SEQ ID NO:227) (即,GGGSGGGS (SEQ ID NO:227)) 或5XGGGS (SEQ ID NO:228)。接头可以在铁蛋白的N或C末端。

[0138] 在一些实施方案中,接头的长度为2、3、4、5、6、7、8、9或10个氨基酸。在一些实施方案中,接头的长度为约2-4、2-6、2-8、2-10、2-12或2-14个氨基酸。在一些实施方案中,接头的长度为至少15个氨基酸。在一些实施方案中,接头的长度为至少25个氨基酸。在一些实施方案中,接头的长度为至少30个氨基酸。在一些实施方案中,接头的长度为至少35个氨基酸。在一些实施方案中,接头的长度为至少40个氨基酸。在一些实施方案中,接头的长度小于或等于60个氨基酸。在一些实施方案中,接头的长度小于或等于50个氨基酸。在一些实施方案中,接头的长度为约16、28、40、46或47个氨基酸。在一些实施方案中,接头是柔性的。在一些实施方案中,接头包含半胱氨酸,例如以用作用于缀合免疫刺激部分(例如,佐剂)的位点;包含半胱氨酸的示例性接头提供为SEQ ID NO:225。在一些实施方案中,接头包含与SEQ ID NO:225具有至少75%、80%、85%、90%或95%同一性的序列,并且进一步包含对应于SEQ ID NO:225中的半胱氨酸的半胱氨酸。在一些实施方案中,接头包含至少25个氨基酸(例如,25至60个氨基酸),其中半胱氨酸位于在自N末端的第8个氨基酸到自C末端的第8个氨基酸范围内的位置处,或位于接头的中心残基或键的10个氨基酸之内。

[0139] 在一些实施方案中,接头包含甘氨酸(G)和/或丝氨酸(S)氨基酸。在一些实施方案中,接头包含以下项或由以下项组成:甘氨酸(G)、丝氨酸(S)、天冬酰胺(N)和/或丙氨酸(A)氨基酸以及任选的如本文所讨论的半胱氨酸。在一些实施方案中,接头包含与SEQ ID NO:222具有至少80%、85%、90%、95%、97%、98%、99%或100%同一性的氨基酸序列。在一些

实施方案中,接头是GGGSGGGSGGGSG (SEQ ID NO:220)、GGSGSNSSASSGASSGGSGGGSG (SEQ ID NO:221)、GGSGSAS SGASASGSSNGSGSGSNSSASSGASSGGSGGGSG (SEQ ID NO:222) 或GS。在一些实施方案中,接头是FR1 (SEQ ID NO:223) 或FR2 (SEQ ID NO:224)。

[0140] 在一些实施方案中,铁蛋白包括具有牛蛙铁蛋白的氨基末端延伸区的幽门螺杆菌铁蛋白(其将被称为杂合铁蛋白)。在一些实施方案中,此杂合铁蛋白形成具有均匀分布在表面上的流感多肽附接位点的多聚体(参见Kanekiyo 2015)。在一些实施方案中,具有杂合铁蛋白的N末端融合蛋白允许流感多肽在铁蛋白纳米颗粒表面上的呈递。在一些实施方案中,铁蛋白在对应于SEQ ID NO:208(杂合铁蛋白,其包含此谷氨酸)的位置13或在SEQ ID NO:209(野生型幽门螺杆菌铁蛋白,其中位置6是异亮氨酸)中的位置6的位置处包含谷氨酸。与牛蛙接头结合时,此谷氨酸被认为可以保留人和牛蛙铁蛋白中发现的保守盐桥(人轻链和牛蛙低级亚基铁蛋白中的6R和14E)。参见Kanekiyo等人,Cell 162,1090-1100 (2015)。

[0141] 在一些实施方案中,流感多肽经由半胱氨酸-凝血酶-组氨酸接头连接至铁蛋白。在一些实施方案中,此接头用于经由点击化学将部分(例如,免疫刺激部分或流感多肽)直接缀合至铁蛋白。包含半胱氨酸-凝血酶-组氨酸接头的示例性序列是SEQ ID NO:218。本文讨论了适合于涉及半胱氨酸-凝血酶-组氨酸接头的缀合反应的点击化学。

[0142] 在一些实施方案中,将包含半胱氨酸作为免疫刺激部分(如佐剂)的缀合位点的接头用于包含缺乏不成对的表面暴露的半胱氨酸的铁蛋白分子的构建体中,或用于包含含有不成对的表面暴露的半胱氨酸的铁蛋白分子的构建体中。

[0143] 在一些实施方案中,构建体不包含接头。在一些实施方案中,构建体包含一个接头。在一些实施方案中,构建体包含两个或多于两个的接头。

4. 结构比对

[0144] 如本文所讨论,可以通过成对或结构比对来鉴定对应于关于给定多肽(例如,幽门螺杆菌铁蛋白)描述的那些的突变位置。结构比对与大型蛋白质家族(如铁蛋白)有关,在大型蛋白质家族中,尽管有相当大的序列变异,但蛋白质共享相似的结构并且家族的许多成员已在结构上进行了表征;并且还可以用于鉴定本文所述的其他多肽(如流感多肽(例如,血凝素))的不同形式中的相应位置。蛋白质数据库(PDB)包括许多铁蛋白的3D结构,包括下文用其登录号列出的那些。

[0145] 2jd6、2jd7-PfFR-激烈火球菌。2jd8-PfFR+Zn。3a68-来自基因SferH4的soFR-大豆。3a9q-来自基因SferH4的soFR(突变体)。3egm、3bvf、3bvi、3bvk、3bvl-HpFR-幽门螺杆菌。5c6f-HpFR(突变体)+Fe。1z4a、1vlg-FR-海栖热袍菌(Thermotoga maritima)。1s3q、1sq3、3kx9-FR-闪烁古生球菌(Archaeoglobus fulgidus)。1krq-FR-空肠弯曲菌(Campylobacter jejuni)。1eum-EcFR-大肠杆菌。4re u-EcFR+Fe。4xgs-EcFR(突变体)+Fe20。4ztt-EcFR(突变体)+Fe20+Fe2+Fe+O2。1qgh-LiFR-英诺克李斯特菌(Listeria innocua)。3qz3-VcFR-霍乱弧菌(Vibrio cholerae)。3vnx-FR-孔石莼(Ulva pertusa)。4ism、4isp、4itt、4itw、4iwj、4iwk、4ixk、3e6s-PnmFR-多列拟菱形藻(Pseudo-nitzschia multiseries)。4zkh、4zkw、4zks、4zl5、4zl6、4zlw、4zmc-PnmFR(突变体)+Fe。1z6o-FR-粉纹夜蛾。4cmy-FR+Fe-花柱绿杆菌(Chlorobaculum tepidum)。铁蛋白轻链(F TL)。11b3、1h96-mFTL-小鼠。1rcc、1rcd、1rci-bFTL+酒石酸盐+Mg。1rce、1rcg-bFTL+酒石酸盐+Mn。3noz、

3np0、3np2、3o7r-hoFTL (突变体) - 马。3o7s、3u90-hoFTL。4v1w-hoFTL-低温EM。3rav、3rd0-hoFTL+巴比妥酸盐。铁蛋白轻+重链:5gn8-hFTH+Ca。

[0146] 结构比对涉及通过以下方式鉴定两个(或更多个)多肽序列中的相应残基:(i)使用第二序列的已知结构对第一序列的结构建模或(ii)比较两者均已知的第一序列和第二序列的结构,并且鉴定第一序列中与第二序列中的目的残基最相似定位的残基。在一些算法中,基于叠加结构中的 α -碳距离最小化(例如,哪组成对 α 碳为比对提供最小的均方根偏差)鉴定出相应的残基。当鉴定非幽门螺杆菌铁蛋白中的对应于关于幽门螺杆菌铁蛋白描述的位置的位置时,幽门螺杆菌铁蛋白可以是“第二”序列。在目的幽门螺杆菌铁蛋白没有已知的可用结构,但相比于幽门螺杆菌铁蛋白与另一种具有已知结构的非幽门螺杆菌铁蛋白紧密相关的情况下,可能最有效的是使用紧密相关的非幽门螺杆菌铁蛋白的已知结构来对目的非幽门螺杆菌铁蛋白建模,然后将该模型与幽门螺杆菌铁蛋白结构进行比较,以鉴定目的铁蛋白中的期望的相应残基。关于结构建模和比对有大量文献,代表性的披露文本包括US 6859736;US 8738343;以及Aslam等人,Electronic Journal of Biotechnology 20 (2016) 9-13中引用的内容。对于基于已知的一种或多种相关结构对结构建模的讨论,参见例如Bordoli等人,Nature Protocols 4 (2009) 1-13及其中引用的参考文献。

5. 免疫刺激部分;佐剂;缀合的流感多肽

[0147] 在一些实施方案中,将流感多肽和/或免疫刺激部分(如佐剂)衔接至铁蛋白的表面暴露的氨基酸或接头。在一些实施方案中,表面暴露的氨基酸是半胱氨酸,例如,由上文讨论的突变产生的半胱氨酸。在一些实施方案中,表面暴露的氨基酸是赖氨酸、天冬氨酸或谷氨酸。使用戊二醛(用于将赖氨酸与带氨基的接头或部分缀合)或碳二亚胺(例如用于将天冬氨酸或谷氨酸与带氨基的接头或部分或将赖氨酸与带羧基的接头或部分缀合的1-环己基-3-(2-吗啉-4-基-乙基)碳二亚胺或1-乙基-3-(3-二甲基-氨基丙基)碳二亚胺(EDC;EDAC))的缀合程序例如描述于可从万维网springer.com获得的Holtzhauer, M., Basic Methods for the Biochemical Lab, Springer 2006, ISBN 978-3-540-32785-1的第四章中。

[0148] 在一些实施方案中,将免疫刺激部分(如佐剂)衔接至铁蛋白的表面暴露的氨基酸。在一些实施方案中,将多于一个免疫刺激部分(如佐剂)衔接至铁蛋白的表面暴露的氨基酸。在一些实施方案中,将二十四免疫刺激部分衔接至铁蛋白多聚体或颗粒(例如,幽门螺杆菌铁蛋白颗粒中每个单体有一个部分)。在具有多个衔接至铁蛋白纳米颗粒的免疫刺激部分的实施方案中,所有的免疫刺激部分是相同的。在具有多个衔接至铁蛋白纳米颗粒的免疫刺激部分的实施方案中,所有的免疫刺激部分是不相同的。

a) 免疫刺激部分的类型;佐剂

[0149] 可以衔接至表面暴露的氨基酸(例如半胱氨酸)的任何免疫刺激部分都可以用于根据本公开文本的铁蛋白中。在一些实施方案中,免疫刺激部分是B细胞激动剂。

[0150] 在一些实施方案中,免疫刺激部分不是疏水性的。在一些实施方案中,免疫刺激部分是亲水性的。在一些实施方案中,免疫刺激部分是极性的。在一些实施方案中,免疫刺激部分能够进行氢键结合或离子键结合,例如包含氢键供体、氢键受体、阳离子部分或阴离子部分。如果部分在生理上相关的pH(如pH 6、7、7.4或8)下在水性溶液中将被离子化,则可以认为所述部分是阳离子或阴离子的。

[0151] 在一些实施方案中,免疫刺激部分是佐剂。在一些实施方案中,佐剂包括病原体相关分子模式(PAMP)。在一些实施方案中,佐剂是Toll样受体(TLR)激动剂,或干扰素基因刺激物(STING)激动剂。在一些实施方案中,佐剂激活B和/或T细胞中的TLR信号传导。在一些实施方案中,佐剂调节适应性免疫应答。

(1) TLR2激动剂

[0152] 在一些实施方案中,免疫刺激部分是TLR2激动剂。在一些实施方案中,免疫刺激部分刺激TLR2信号传导。在一些实施方案中,免疫刺激部分是TLR2的合成小分子配体。在一些实施方案中,免疫刺激部分是TLR2信号传导的合成小分子激动剂。

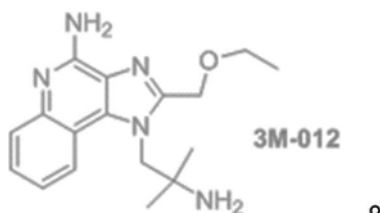
[0153] 在一些实施方案中,TLR2激动剂是PAM2CSK4、FSL-1或PAM3CSK4。

(2) TLR7/8激动剂

[0154] 在一些实施方案中,免疫刺激部分是TLR7和/或TLR8激动剂(即,TLR7和TLR8中至少一者的激动剂)。在一些实施方案中,免疫刺激部分刺激TLR7和/或TLR8信号传导。在一些实施方案中,免疫刺激部分是TLR7和/或TLR8的合成小分子配体。在一些实施方案中,免疫刺激部分是TLR7和/或TLR8信号传导的合成小分子激动剂。

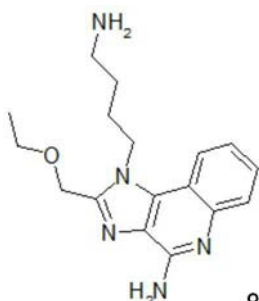
[0155] 在一些实施方案中,TLR7和/或TLR8激动剂是单链的(ssRNA)。在一些实施方案中,TLR7和/或TLR8激动剂是咪唑并喹啉。在一些实施方案中,TLR7和/或TLR8激动剂是核苷类似物。

[0156] 在一些实施方案中,TLR7和/或TLR8激动剂是咪唑并喹啉胺Toll样受体(TLR)激动剂,如3M-012(3M Pharmaceuticals)。游离3M-012的结构是:



应当理解的是,可以通过以下方式将免疫刺激部分(如3M-012)或本文所讨论的任何部分缀合至铁蛋白:用与本文所述的铁蛋白例如在表面暴露的半胱氨酸的硫处的键或与附接至这个硫的接头的键取代所述部分的适当的外围原子(例如氢)。因此,当缀合至铁蛋白时,免疫刺激部分的结构将与游离分子的结构略有不同。

[0157] 在一些实施方案中,TLR7和/或TLR8激动剂是SM 7/8a。游离SM 7/8a的结构是:



[0158] 参见,例如Nat Biotechnol.2015年11月;33(11):1201-10.doi:10.1038/nbt.3371。

(3) TLR9激动剂

[0159] 在一些实施方案中,免疫刺激部分是TLR9激动剂。在一些实施方案中,免疫刺激部分刺激TLR9信号传导。在一些实施方案中,免疫刺激部分是TLR9的合成小分子配体。在一些实施方案中,免疫刺激部分是TLR9信号传导的合成小分子激动剂。

[0160] 在一些实施方案中,TLR9激动剂是CpG寡脱氧核苷酸(ODN)。在一些实施方案中,TLR9激动剂是未甲基化的CpG ODN。在一些实施方案中,CpG ODN包含部分或完整的硫代磷酸酯(PS)主链,而不是普通DNA中发现的天然磷酸二酯(PO)主链。

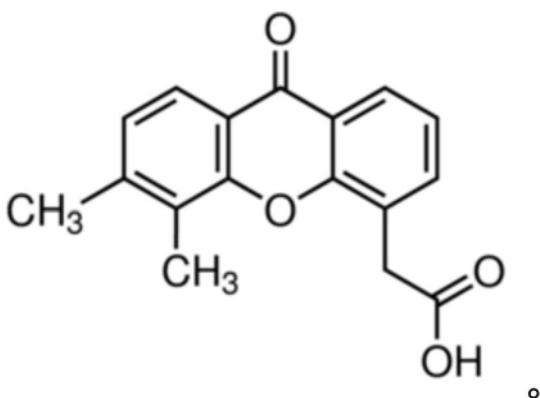
[0161] 在一些实施方案中,CpG ODN是B类ODN,其包含一个或多个含有5'嘌呤(Pu)-嘧啶(Py)-C-G-Py-Pu 3'的6聚体CpG基序;具有完全硫代磷酸化(即PS改性)的主链;并且长度为18-28个核苷酸。在一些实施方案中,CpG ODN包含SEQ ID NO:210的序列,任选地包含在主链中的硫代磷酸酯连接。

[0162] 在一些实施方案中,TLR9激动剂包含免疫刺激序列(ISS)。在一些实施方案中,TLR9激动剂是ISS-1018(Dynavax)(SEQ ID NO:210)。

(4) STING激动剂

[0163] 在一些实施方案中,免疫刺激部分是STING(干扰素基因蛋白的刺激物,也称为内质网IFN刺激物)激动剂。在一些实施方案中,免疫刺激部分刺激STING信号传导。在一些实施方案中,免疫刺激部分是STING的合成小分子配体。在一些实施方案中,免疫刺激部分是STING信号传导的合成小分子激动剂。

[0164] 在一些实施方案中,STING激动剂是环状二核苷酸(CDN)。参见,例如Danilchanka等人,Ce11 154:962-970(2013)。示例性CDN包括cdA、cdG、cAMP-cGMP和2'-5',3'-5'cGAMP(对于结构,参见Danilchanka等人)。STING激动剂还包括合成激动剂,如DMXAA



b) 缀合的流感多肽

[0165] 在一些实施方案中,流感多肽缀合至铁蛋白的表面暴露的氨基酸。在一些实施方案中,流感多肽单独地具有抗原性,而在一些实施方案中,流感多肽由于其与铁蛋白的缔合而具有抗原性。流感多肽可以是本文所述的流感多肽中的任一种。

c) 缀合

[0166] 在一些实施方案中,表面暴露的半胱氨酸(例如,由本文所述的突变产生)或衔接至铁蛋白(例如,在铁蛋白的N末端)的肽接头中的半胱氨酸用于将免疫刺激部分(如佐剂)或流感多肽缀合至铁蛋白。在一些实施方案中,接头与这种半胱氨酸缀合,可以随后将所述接头缀合至免疫刺激部分(如佐剂)或流感多肽。在一些实施方案中,这种半胱氨酸产生用于缀合反应以衔接佐剂、接头或流感多肽的化学处理。在一些实施方案中,产生了生物缀合

物,其中在还原这种半胱氨酸后将免疫刺激部分(如佐剂)或流感多肽连接至铁蛋白。在一些实施方案中,半胱氨酸是不成对的表面暴露的半胱氨酸,即所述半胱氨酸缺乏适当位置的伴侣半胱氨酸来形成二硫键。在一些实施方案中,半胱氨酸是包含游离硫醇侧链的不成对半胱氨酸。

(1) 缀合化学的类型

[0167] 可以使用任何类型的化学,例如经由表面暴露的氨基酸(如半胱氨酸)或另一种氨基酸(如Lys、Glu或Asp)的反应,来将免疫刺激部分(如佐剂)或流感多肽缀合至铁蛋白。

[0168] 在一些实施方案中,使用点击化学进行缀合。如本文所用,“点击化学”是指彼此快速且选择性地反应(即,“点击”)的一对官能团之间的反应。在一些实施方案中,点击化学可以在温和的水性条件下进行。在一些实施方案中,点击化学反应利用铁蛋白表面上的半胱氨酸(如由表面暴露的氨基酸突变产生的半胱氨酸)来使用可与所述半胱氨酸反应的官能团进行点击化学。

[0169] 满足点击化学标准的多种反应在本领域中是已知的,并且本领域技术人员可以使用许多公布的方法中的任何一种(参见,例如Hein等人,Pharm Res 25(10):2216-2230(2008))。可以使用众多可商购的点击化学试剂,如来自Sigma Aldrich、Jena Bioscience或Lumiprobe的那些。在一些实施方案中,如以下实施例所述那样使用点击化学进行缀合。

[0170] 在一些实施方案中,点击化学反应在铁蛋白还原之后发生。

[0171] 在一些实施方案中,点击化学可以是1步式点击反应。在一些实施方案中,点击化学可以是2步式点击反应。

[0172] 在一些实施方案中,一个或多个反应包括无金属点击化学。在一些实施方案中,一个或多个反应包括硫醇-马来酰亚胺和/或二硫化物交换。

无金属点击化学

[0173] 可以将无金属点击化学用于缀合反应以避免蛋白质的潜在氧化。无金属点击化学已被用于形成抗体缀合物(参见van Geel等人,Bioconjugate Chem.2015,26,2233-2242)。

[0174] 在一些实施方案中,在反应中使用无金属点击化学以将佐剂附接至铁蛋白。在一些实施方案中,在反应中使用无铜缀合以将佐剂附接至铁蛋白。在一些实施方案中,无金属点击化学使用双环[6.1.0]壬炔(BCN)。在一些实施方案中,无金属点击化学使用二苯并氮杂环辛炔(DBCO)。在一些实施方案中,BCN或DBCO与叠氮化物基团反应。

[0175] DBCO在不存在催化剂的情况下经由应变促进的点击反应对叠氮化物基团具有高特异性,从而产生高产率的稳定三唑。在一些实施方案中,DBCO在不存在铜催化剂的情况下与叠氮化物反应。

[0176] 在一些实施方案中,在1步式点击反应中使用无金属点击化学。在一些实施方案中,在2步式点击反应中使用无金属点击化学。

硫醇-马来酰亚胺和二硫化物交换

[0177] 本文所述的铁蛋白可以包含含有硫醇(也称为巯基)的半胱氨酸,其可用于与巯基反应性化学基团反应(或其可通过还原而变为可用的)。因此,半胱氨酸允许化学选择性修饰以将免疫刺激部分(如佐剂)添加至铁蛋白。在碱性条件下,半胱氨酸将被去质子化以产生硫醇盐亲核体,所述硫醇盐亲核体可以与软亲电体(如马来酰亚胺和碘乙酰胺)反应。半

胱氨酸与马来酰亚胺或碘乙酰胺的反应产生碳-硫键。

[0178] 在一些实施方案中, 巯基反应性化学基团与铁蛋白的表面暴露的半胱氨酸或铁蛋白接头中的半胱氨酸反应。在一些实施方案中, 巯基反应性化学基团是卤代乙酰基、马来酰亚胺、氮丙啶、丙烯酰基、芳基化剂、乙烯基砜、吡啶基二硫化物或TNB-硫醇。

[0179] 在一些实施方案中, 巯基反应性化学基团通过烷基化(即形成硫醚键)与半胱氨酸的巯基缀合。在一些实施方案中, 巯基反应性化学基团通过二硫化物交换(即形成二硫键)与半胱氨酸的巯基缀合。

[0180] 在一些实施方案中, 将免疫刺激部分(如佐剂)缀合至铁蛋白的反应是硫醇-马来酰亚胺反应。

[0181] 在一些实施方案中, 巯基反应性化学基团是马来酰亚胺。在一些实施方案中, 马来酰亚胺与半胱氨酸的反应导致形成稳定的硫酯连接, 例如不可逆的硫酯连接。在一些实施方案中, 马来酰亚胺不与铁蛋白中的酪氨酸、组氨酸或甲硫氨酸反应。在一些实施方案中, 未反应的马来酰亚胺在反应结束时通过添加例如过量的游离硫醇来淬灭。

[0182] 在一些实施方案中, 使免疫刺激部分(如佐剂)与铁蛋白缀合的反应是硫醇-二硫化物交换, 也称为二硫化物互换。在一些实施方案中, 反应涉及形成包含一部分原始二硫化物的混合二硫化物。在一些实施方案中, 原始二硫化物是通过表面暴露的氨基酸的突变或N末端接头的添加而在铁蛋白中引入的半胱氨酸。

[0183] 在一些实施方案中, 巯基反应性化学基团是吡啶基二硫醇。在一些实施方案中, 巯基反应性化学基团是TNB-硫醇基团。

(2) 接头

[0184] 在一些实施方案中, 免疫刺激部分(如佐剂)或流感多肽经由与表面暴露的氨基酸(如半胱氨酸)共价结合的接头附接至铁蛋白。在一些实施方案中, 接头包含聚乙二醇, 例如PEG接头。在一些实施方案中, 聚乙二醇(例如PEG)接头增加了与免疫刺激部分(如佐剂)连接的铁蛋白的水溶性和连接效率。PEG接头的长度在2至18个PEG之间, 例如PEG4、PEG5、PEG6、PEG7、PEG8、PEG9、PEG10、PEG11、PEG12、PEG13、PEG14、PEG15、PEG16、PEG17和PEG18。

[0185] 在一些实施方案中, 接头包含马来酰亚胺。在一些实施方案中, 接头包含免疫刺激部分(ISM)-接头-马来酰亚胺的组分。在一些实施方案中, 通过马来酰亚胺与铁蛋白的半胱氨酸的反应, 以1步式点击化学反应将ISM-接头-马来酰亚胺缀合至铁蛋白。在一些实施方案中, 佐剂-接头-马来酰亚胺的ISM是SM7/8a。在一些实施方案中, ISM-接头-马来酰亚胺的接头是PEG4。在一些实施方案中, ISM-接头-马来酰亚胺是SM7/8a-PEG4-马来酰亚胺。

[0186] 在一些实施方案中, 用下述接头使用2步式点击化学方案, 所述接头在一端包含巯基反应性化学基团, 并且在另一端包含胺反应性基团。在这种2步式点击化学方案中, 巯基反应性化学基团与铁蛋白的半胱氨酸反应, 而胺反应性基团与附接至ISM的试剂反应。以这种方式, ISM经由一组2种点击化学试剂缀合至铁蛋白。

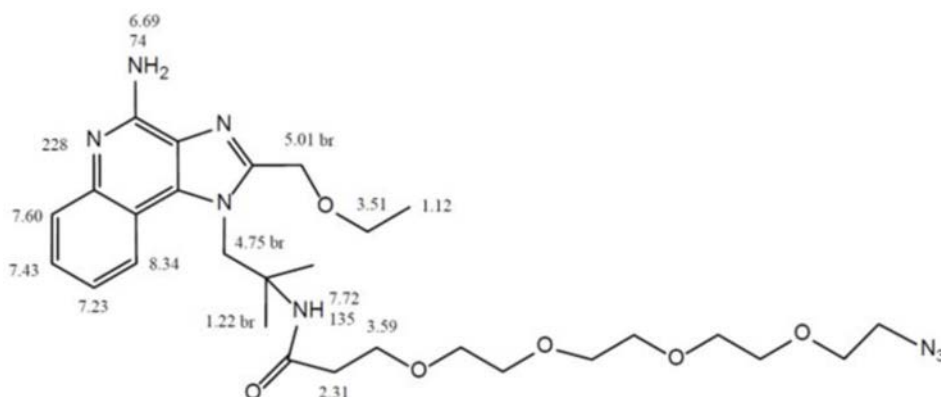
[0187] 在2步式点击化学方案的一些实施方案中, 巯基反应性化学基团是马来酰亚胺。在2步式点击化学方案的一些实施方案中, 马来酰亚胺与通过表面暴露的氨基酸的突变或N末端接头的添加而引入铁蛋白中的半胱氨酸反应。

[0188] 在2步式点击化学方案的一些实施方案中, 胺反应性基团是DBCO。在2步式点击化学方案的一些实施方案中, DBCO与附接至ISM的叠氮化物基团反应。

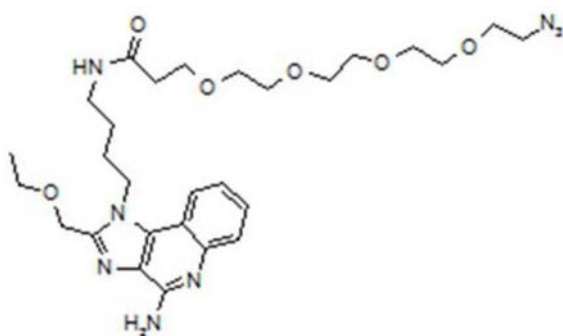
[0189] 在一些实施方案中,使用马来酰亚胺-接头-DBCO。在一些实施方案中,在铁蛋白还原后,将马来酰亚胺-接头-DBCO缀合至铁蛋白。在一些实施方案中,通过在这第一步中马来酰亚胺与铁蛋白的半胱氨酸的反应,将马来酰亚胺-接头-试剂缀合至铁蛋白。在一些实施方案中,DBCO用于与附接至叠氮化物的ISM连接。在一些实施方案中,偶联至叠氮化物的ISM是ISS-1018。在一些实施方案中,偶联至叠氮化物的佐剂是3M-012或CpG。

[0190] 在一些实施方案中,将具有反应性基团的接头添加至ISM。在一些实施方案中,接头是PEG4-叠氮化物接头或PEG4-马来酰亚胺接头。

[0191] 在一些实施方案中,PEG4-叠氮化物接头缀合至3M-012。缀合至PEG4-叠氮化物接头的3M-012的示例性结构为:



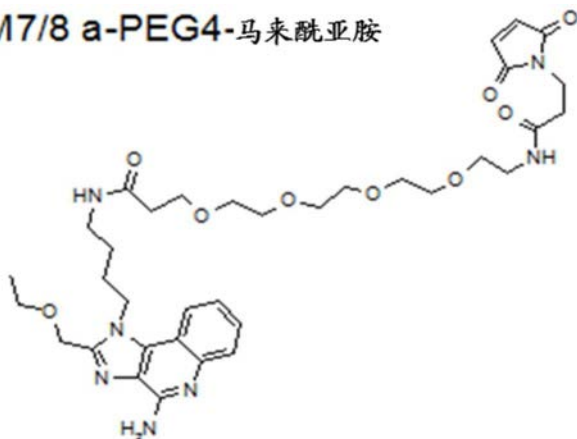
[0192] 在一些实施方案中,PEG4-叠氮化物接头缀合至SM7/8a。缀合至PEG4-叠氮化物接头的SM7/8a的示例性结构为:



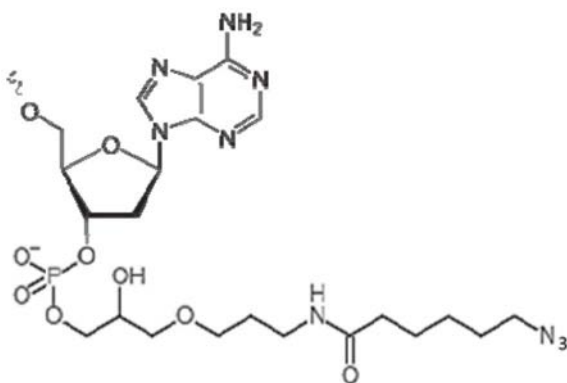
SM7/8a -PEG4-叠氮化物

[0193] 在一些实施方案中,PEG4-马来酰亚胺接头缀合至SM7/8a。缀合至PEG4-马来酰亚胺接头的SM7/8a的示例性结构为:

SM7/8 a-PEG4-马来酰亚胺



[0194] 在一些实施方案中,叠氮化物基团缀合至ISS-1018。缀合至NHS酯-叠氮化物接头的ISS-1018的示例性结构为:



C. 示例性组合物、试剂盒、核酸、用途和方法

[0195] 在一些实施方案中,提供了组合物,所述组合物包含本文所述的抗原性多肽中的任何一种或多种和药学上可接受的媒介物、佐剂或赋形剂。

[0196] 在一些实施方案中,本发明提供了针对流感感染免疫受试者的方法。本发明进一步提供了在受试者中引发针对流感的免疫应答的方法。在一些实施方案中,本方法包括向受试者给予有效量的本文所述的药物组合物。在一些实施方案中,本方法包括向受试者给予有效量的本文所述的抗原性流感-铁蛋白多肽或纳米颗粒。

[0197] 在一些实施方案中,将本文所述的抗原性多肽或组合物给予至受试者(如人),以产生针对将来的流感感染的保护性免疫应答。在一些实施方案中,给予了包含SEQ ID NO: 1-43中任一个的抗原性多肽。

[0198] 在一些实施方案中,保护性免疫应答降低了住院的发生率。在一些实施方案中,保护性免疫应答降低了实验室确认的流感感染的发生率。

[0199] 在一些实施方案中,可以向个体给予多于一种抗原性多肽以针对多种流感株系免疫,其中抗原性多肽的HA或NA多肽部分不同。给予可以是同时的或顺序的。在一些实施方案中,给予不同流感株系的两种HA或NA多肽。在一些实施方案中,给予不同流感株系的三种HA或NA多肽。在一些实施方案中,给予不同流感株系的四种HA或NA多肽。在一些实施方案中,给予不同流感株系的五种HA或NA多肽。在一些实施方案中,给予不同流感株系的多于五种HA或NA多肽。

1. 受试者

[0200] 在一些实施方案中,受试者是哺乳动物。在一些实施方案中,受试者是人。

[0201] 在一些实施方案中,受试者是成人(大于或等于18岁)。在一些实施方案中,受试者是儿童或青少年(小于18岁)。在一些实施方案中,受试者是老年人(大于60岁)。在一些实施方案中,受试者是非老年人(大于或等于18岁且小于或等于60岁)。

[0202] 在一些实施方案中,向受试者给予多于一次的组合物给予。在一些实施方案中,加强免疫给予改善了免疫应答。

[0203] 在一些实施方案中,本文所述的抗原性多肽或组合物中的任何一种或多种用于在哺乳动物,如灵长类动物(例如,非人灵长类动物,如猴子(例如,猕猴,如恒河猴或食蟹猴)或猿)、啮齿动物(例如,小鼠或大鼠)或驯养的哺乳动物(例如,犬、兔、猫、马、绵羊、牛、山羊、骆驼或驴)中使用。在一些实施方案中,本文所述的抗原性多肽或组合物中的任何一种或多种用于在鸟类,如禽类(例如,鸡、火鸡、鸭、鹅、豚鼠或天鹅)中使用。

2. 佐剂

[0204] 如本文所述,佐剂可以经由表面暴露的氨基酸例如半胱氨酸缀合至铁蛋白。非缀合的佐剂也可以与本文所述的抗原性铁蛋白多肽一起给予至受试者。在一些实施方案中,与在不伴有佐剂的情况下给予单独的流感多肽或单独的抗原性铁蛋白多肽相比,与抗原性铁蛋白多肽一起给予佐剂在受试者中产生更高效价的针对流感多肽的抗体。佐剂可以促进对抗原性多肽的更早、更强效或更持久的免疫应答。

[0205] 在一些实施方案中,组合物包含一种佐剂。在一些实施方案中,组合物包含多于一种佐剂。在一些实施方案中,组合物不包含佐剂。

[0206] 在一些实施方案中,佐剂包含铝。在一些实施方案中,佐剂是磷酸铝。在一些实施方案中,佐剂是明矾(Alyhydrogel' 85 2%;Brenntag-目录号21645-51-2)。

[0207] 在一些实施方案中,佐剂是有机佐剂。在一些实施方案中,佐剂是油基佐剂。在一些实施方案中,佐剂是水包油纳米乳剂。

[0208] 在一些实施方案中,佐剂包含角鲨烯。在一些实施方案中,包含角鲨烯的佐剂是Ribi(Sigma adjuvant system目录号S6322-1vl)、AddavaxTM MF59、AS03或AF03(参见US9703095)。在一些实施方案中,包含角鲨烯的佐剂是纳米乳剂。

[0209] 在一些实施方案中,佐剂包含聚丙烯酸聚合物(PAA)。在一些实施方案中,包含PAA的佐剂是SPA09(参见WO 2017218819)。

[0210] 在一些实施方案中,佐剂包含非可代谢油。在一些实施方案中,佐剂是不完全弗氏佐剂(IFA)。

[0211] 在一些实施方案中,佐剂包含非可代谢油和杀死的结核分枝杆菌。在一些实施方案中,佐剂是完全弗氏佐剂(CFA)。

[0212] 在一些实施方案中,佐剂是脂多糖。在一些实施方案中,佐剂是单磷酰A(MPL或MPLA)。

3. 药物组合物

[0213] 在各种实施方案中,提供了药物组合物,所述药物组合物包含本文所述的抗原性铁蛋白多肽和/或相关实体。在一些实施方案中,药物组合物是能够引发免疫应答(如针对病原体的保护性免疫应答)的免疫原性组合物(例如,疫苗)。

[0214] 例如,在一些实施方案中,药物组合物可以包含以下项中的一种或多种:(1) 抗原

性铁蛋白蛋白,所述抗原性铁蛋白蛋白包含(i)用半胱氨酸置换表面暴露的氨基酸的突变和(ii)流感多肽;(2)抗原性铁蛋白蛋白,所述抗原性铁蛋白蛋白包含(i)用半胱氨酸置换表面暴露的氨基酸的突变和连接至所述半胱氨酸的免疫刺激部分;和(ii)流感多肽;(3)抗原性铁蛋白蛋白,所述抗原性铁蛋白蛋白包含(i)表面暴露的半胱氨酸,(ii)在铁蛋白蛋白的N末端的肽接头,和(iii)在肽接头的N末端的流感多肽;(4)抗原性铁蛋白蛋白,所述抗原性铁蛋白蛋白包含:(i)用半胱氨酸置换表面暴露的氨基酸的突变和连接至所述半胱氨酸的免疫刺激部分,(ii)用非半胱氨酸氨基酸置换在幽门螺杆菌铁蛋白的位置31处的内部半胱氨酸的突变,或用非半胱氨酸氨基酸置换如通过成对或结构比对确定的非幽门螺杆菌铁蛋白中与位置31类似的位置处的内部半胱氨酸的突变,(iii)用非天冬酰胺氨基酸置换表面暴露的天冬酰胺的突变,和(iv)流感多肽;或(5)包含前述铁蛋白蛋白中的任一种的铁蛋白颗粒。

[0215] 在一些实施方案中,本发明提供了药物组合物,所述药物组合物包含与本文所述的抗原性多肽相关的抗体或其他药剂。在一个实施方案中,药物组合物包含与本文所述的抗原性多肽结合和/或竞争的抗体。可替代地,抗体可以识别包含本文所述的抗原性多肽的流感多肽组分的病毒颗粒或细菌。

[0216] 在一些实施方案中,将如本文所述的药物组合物单独给予或与一种或多种增强免疫应答的药剂(例如,以上所述的佐剂)组合给予。在一些实施方案中,药物组合物进一步包含如上所述的佐剂。

[0217] 在一些实施方案中,药物组合物进一步包含药学上可接受的载体或赋形剂。如本文所用,术语“载体”是指与药物组合物一起给予的稀释剂、佐剂、赋形剂或媒介物。在示例性实施方案中,载体可以包括无菌液体,例如像水和油,包括石油、动物、植物或合成起源的油,例如像花生油、大豆油、矿物油、芝麻油等。在一些实施方案中,载体是或包括一种或多种固体组分。药学上可接受的载体还可包括但不限于盐水、缓冲盐水、右旋糖、甘油、乙醇及其组合。如本文所用,赋形剂是可以包含在药物组合物中例如以提供或有助于所希望的稠度或稳定作用的任何非治疗剂。合适的药物赋形剂包括但不限于淀粉、葡萄糖、乳糖、蔗糖、明胶、麦芽、稻、面粉、白垩、硅胶、硬脂酸钠、单硬脂酸甘油酯、滑石、氯化钠、脱脂乳粉、甘油、丙烯、二醇、水、乙醇等。在各种实施方案中,药物组合物是无菌的。

[0218] 在一些实施方案中,药物组合物含有少量的润湿剂或乳化剂、或pH缓冲剂。在一些实施方案中,药物组合物可以包含多种添加剂(如稳定剂、缓冲液或防腐剂)中的任何一种。此外,可以包含辅助剂、稳定剂、增稠剂、润滑剂和着色剂。

[0219] 在各种实施方案中,可以将药物组合物配制或制成适合任何所期望的给予方式。例如,药物组合物可以采取以下形式:溶液、悬浮液、乳剂、滴剂、片剂、丸剂、球剂、胶囊、含有液体的胶囊、明胶胶囊、粉末、缓释配制品、栓剂、乳剂、气雾剂、喷雾剂、悬浮液、冻干粉、冷冻悬浮液、干燥粉或任何其他适合使用的形式。在药剂的配制和生产中的一般考虑可以见于例如Remington's Pharmaceutical Sciences,第19版,Mack Publishing Co.,宾夕法尼亚州伊斯顿,1995(通过引用并入本文)中。

[0220] 可以经由任何给予途径来给予药物组合物。给予途径包括例如口服、皮内、肌内、腹膜内、静脉内、皮下、鼻内、粘膜、硬膜外、舌下、鼻内、脑内、阴道内、透皮、经直肠、通过气管内滴注、支气管滴注、吸入、或局部方式。给予可为局部或全身的。在一些实施方案中,给

予是口服进行的。在另一个实施方案中,给予是通过肠胃外注射。在一些情况下,给予导致本文所述的抗原性铁蛋白多肽释放到血流中。给予方式可以由从业者决定。

[0221] 在一些实施方案中,药物组合物适合于肠胃外给予(例如静脉内、肌内、腹膜内和皮下)。可以将此类组合物配制成例如溶液、悬浮液、分散体、乳剂等。它们也可以以无菌固体组合物(例如冻干的组合物)的形式生产,所述无菌固体组合物可以在临使用前溶解或悬浮于无菌可注射介质中。例如,肠胃外给予可以通过注射来实现。在此类实施方案中,注射剂以常规形式制备,即作为液体溶液或悬浮液、适合于在注射前溶解或悬浮于液体中的固体形式、或作为乳剂。在一些实施方案中,注射溶液和悬浮液由无菌粉末、冻干粉末或颗粒制备。

[0222] 在另一个实施方案中,将药物组合物配制成用于通过吸入递送(例如,用于直接递送至肺和呼吸系统)。例如,组合物可以采取鼻喷雾剂或任何其他已知的气雾剂制品的形式。在一些实施方案中,用于吸入或气雾剂递送的制剂包含多个颗粒。在一些实施方案中,此类制剂可以具有约1、约2、约3、约4、约5、约6、约7、约8、约9、约10、约11、约12或约13微米的平均粒度。在一些实施方案中,用于吸入或气雾剂递送的制剂被配制为干粉。在一些实施方案中,用于吸入或气雾剂递送的制剂例如通过包含润湿剂而被配制为湿粉。在一些实施方案中,润湿剂选自水、盐水或其他处于生理pH的液体。

[0223] 在一些实施方案中,将根据本发明的药物组合物以滴剂的形式给予至鼻腔或颊腔。在一些实施方案中,剂量可以包含多滴(例如1-100、1-50、1-20、1-10、1-5滴等)。

[0224] 可以将本发明的药物组合物以适合于实现所期望结果的任何剂量给予。在一些实施方案中,所期望的结果是诱导针对病原体的持久的适应性免疫应答,所述病原体如存在于组合物中的抗原性铁蛋白多肽中的流感多肽的来源。在一些实施方案中,所期望的结果是降低一种或多种感染症状的强度、严重程度、频率和/或延迟一种或多种感染症状的发作。在一些实施方案中,所期望的结果是抑制或预防感染。所需的剂量将根据以下而在受试者之间有所不同:受试者的物种、年龄、体重和一般状况,被预防或治疗的感染的严重程度,所使用的特定组合物及其给予方式。

[0225] 在一些实施方案中,将根据本发明的药物组合物以单一剂量或多个剂量给予。在一些实施方案中,将药物组合物在不同日期以多个剂量给予(例如初免-加强免疫策略)。在一些实施方案中,将药物组合物作为加强免疫方案的一部分给予。

[0226] 在各种实施方案中,将药物组合物与一种或多种另外的治疗剂共同给予。如果治疗剂的给予的时间安排使得另外的治疗剂和药物组合物中的一种或多种活性成分的药理活性在时间上重叠,从而发挥组合的治疗作用,则共同给予不需要同时给予治疗剂。通常,每种药剂将以针对所述药剂确定的剂量和时间表来给予。

4. 核酸/mRNA

[0227] 还提供了核酸,所述核酸编码本文所述的抗原性流感-铁蛋白多肽。在一些实施方案中,核酸是mRNA。出于本公开文本的目的,任何能够经历翻译而产生多肽的核酸被认为是mRNA。

5. 试剂盒

[0228] 本文还提供了试剂盒,所述试剂盒包括本文所述的一种或多种抗原性多肽、核酸、抗原性铁蛋白颗粒、组合物或药物组合物。在一些实施方案中,试剂盒进一步包括溶剂、溶

液、缓冲液、说明书或干燥剂中的一种或多种。

[0229] 此描述和示例性实施方案不应被视为是限制性的。为了本说明书和所附权利要求的目的,除非另有指示,否则在说明书和权利要求中使用的所有表达数量、百分比或比例的数字和其他数值应被理解为在所有情况下被术语“约”修饰至已无法将其如此修饰的程度。“约”指示实质上不影响所述主题的特性的变化程度,例如在10%、5%、2%或1%之内。因此,除非有相反的指示,否则以下说明书和所附权利要求中阐述的数值参数是近似值,所述近似值可以根据试图获得的所需特性而变化。在最低限度并且不试图限制对权利要求范围的等价范围的原则的应用,每个数值参数均应当至少考虑报告的有效数字的数量和通过应用普通四舍五入技术来解读。

[0230] 注意,如本说明书和所附权利要求所用,单数形式“一个/种(a、an)”和“所述(the)”以及任何词语的任何单数使用包括复数个指示物,除非明确地并且清楚地限于一个/种指示物。如本文所用,术语“包括”及其语法变体旨在是非限制性的,使得列表中项目的详述不排除可以取代或添加到所列项目的其他类似项目。除非上下文另有指示,否则术语“或”以包括性含义使用,即等同于“和/或”。

表 1 (序列表): 序列的描述

描述	序列	SEQ ID NO
对于 SEQ ID NO: 1-44 的说明: • <u>前导序列以下划线表示</u> • HA - <u>粗体</u> • TEV - <u>波浪形下划线和斜体</u> • 接头 - <u>双下划线</u> • Bf Ferr 序列 - 正常文本 • pFerr 序列 - <u>波浪形下划线</u> • 用于组合的“Cys”以<u>加粗</u>、<u>粗体</u>、<u>斜体</u>和<u>波浪形下划线</u>表示 • <u>Y98F 突变</u>的 F 以<u>粗体</u>、<u>下划线</u>和<u>斜体</u>表示 • 对于 H5 纳米颗粒的 PORET (SEQ ID NO: 229) (单碱基突变) - 以斜体表示		
SIB 6001: HA(NC99 Y98F)pFerr N19Q/C31S/S111C	MKAKLLVLLCTFTATYADTICIGYHANNSTDVDTVLEKNVTVTSHVNLLDSDSHNGKLCCLKGIAPLQLGNCVAGWILGNPECELLISKESNSYIVETPNPENGTCFPGYFADYEELREQLSSVS SFERFEIPFKESSWPNHTVTGVSASCSHNGKSSFYRNLLMLTGKNGLYPNLSKSYNNKEKEVLVINGVHHPPNIGNQALYHTENAYSVVSSHYSRRFTPEIAKRPKVRDQEGRINYTWTLLEP GDTIIFEANGNLIAFWYAFALSRGFGSGIIITSNAPMDECDKACQTPQGAINSLSLFFQNVHPTTIGECPCYVRSAKLRMTGLRNIPORETRGLFGA TAGFTGGWTGAVDGTWGYHHQNEQGGGYA ADQKSTQNAINGITNKVNSVIERKMTQFVAVGKEFNKLERRMENLNKKVDDGFLDIWTYNAELLVLLNERTLDFHDSNVKNLYEKVKSQLKNNNAKEIGNGCFEFYHKCNNECMESVKNGTYDYPK YSEESKLNREKIDSGEDLIKLINQVKNKEMQSSNLYMSMSWSYTHSLDGGAGLEFEDHAAEEYEHAKKLLIIELENNNVQVLTISIAPBEHKEEGITQIFOKAYEHEQHISESINNIVDHAIKCQDH ATENELQWVAEQHEEVLEFKDILLKIELIGNENHGLYADQXVKGIKSRKSGS	1
SIB 6243: HA(NC99 Y98F)pFerr N19Q/C31S/S111C	MKAKLLVLLCTFTATYADTICIGYHANNSTDVDTVLEKNVTVTSHVNLLDSDSHNGKLCCLKGIAPLQLGNCVAGWILGNPECELLISKESNSYIVETPNPENGTCFPGYFADYEELREQLSSVS SFERFEIPFKESSWPNHTVTGVSASCSHNGKSSFYRNLLMLTGKNGLYPNLSKSYNNKEKEVLVINGVHHPPNIGNQALYHTENAYSVVSSHYSRRFTPEIAKRPKVRDQEGRINYTWTLLEP GDTIIFEANGNLIAFWYAFALSRGFGSGIIITSNAPMDECDKACQTPQGAINSLSLFFQNVHPTTIGECPCYVRSAKLRMTGLRNIPORETRGLFGA TAGFTGGWTGAVDGTWGYHHQNEQGGGYA ADQKSTQNAINGITNKVNSVIERKMTQFVAVGKEFNKLERRMENLNKKVDDGFLDIWTYNAELLVLLNERTLDFHDSNVKNLYEKVKSQLKNNNAKEIGNGCFEFYHKCNNECMESVKNGTYDYPK YSEESKLNREKIDGSESVQRQQFSKDIKLLNEQVKNKEMQSSNLYMSMSWSYTHSLDGGAGLEFEDHAAEEYEHAKKLLIIELENNNVQVLTISIAPBEHKEEGITQIFOKAYEHEQHISESINNIV DHAIKCQDHATENELQWVAEQHEEVLEFKDILLKIELIGNENHGLYADQXVKGIKSRKSGS	2

SIB 6244: HA(NC99 Y98F)bpFerr N19Q/C31S	<u>MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLL</u>EDSHNGKLCCLKGIAPIQLGNCVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHVTGVSASCSHNGKSSFYRNLLMTGKNGLYPNLSKSYVNKKEKVLVLMGVHPPNIGNORALYHTENAYSVVSSHYSRRFTPEIAKRPKVDRDQGRINYTYWTLLP GDTIIFEANGNLIAFWYAFALSRFGSGIITSNAPMDECDACQTPQGAINSLSLFFQNVHPTIGECPKYRSAKLRMTGLRNIPORETRGLFGAAGFTIEGGWTGAVDQWGYGHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERRMENLNKKVDGFLDIWYNAELLVLLNERETLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFFFYHKCNNECMESVKNGTYDYPK YSEESKINREKIDGSESQVRQOFSKDIIEKLLNEQVKNEMQSSNLYMCSHNSYTHSLDAGGLETFDHAABEYEHAKKLLIFLNENNVFVQLTISIAPHEKKEGLTQIEOKAYEHEQHLSESINNLY DHAISKDHATENELQWYVAEQHEBEVLEFKOLLDKIELIGNENHGLVADQXVKGIASRKS	3
SIB 6245: HA(NC99 Y98F)bpFerr N19Q/C31S/E12C	<u>MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLL</u>EDSHNGKLCCLKGIAPIQLGNCVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHVTGVSASCSHNGKSSFYRNLLMTGKNGLYPNLSKSYVNKKEKVLVLMGVHPPNIGNORALYHTENAYSVVSSHYSRRFTPEIAKRPKVDRDQGRINYTYWTLLP GDTIIFEANGNLIAFWYAFALSRFGSGIITSNAPMDECDACQTPQGAINSLSLFFQNVHPTIGECPKYRSAKLRMTGLRNIPORETRGLFGAAGFTIEGGWTGAVDQWGYGHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERRMENLNKKVDGFLDIWYNAELLVLLNERETLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFFFYHKCNNECMESVKNGTYDYPK YSEESKINREKIDGSESQVRQOFSKDIIEKLLNEQVKNEMQSSNLYMCSHNSYTHSLDAGGLETFDHAABEYEHAKKLLIFLNENNVFVQLTISIAPHEKKEGLTQIEOKAYEHEQHLSESINNLY DHAISKDHATENELQWYVAEQHEBEVLEFKOLLDKIELIGNENHGLVADQXVKGIASRKS	4
SIB 6246: HA(NC99 Y98F)bpFerr N19Q/C31S/S26C	<u>MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLL</u>EDSHNGKLCCLKGIAPIQLGNCVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHVTGVSASCSHNGKSSFYRNLLMTGKNGLYPNLSKSYVNKKEKVLVLMGVHPPNIGNORALYHTENAYSVVSSHYSRRFTPEIAKRPKVDRDQGRINYTYWTLLP GDTIIFEANGNLIAFWYAFALSRFGSGIITSNAPMDECDACQTPQGAINSLSLFFQNVHPTIGECPKYRSAKLRMTGLRNIPORETRGLFGAAGFTIEGGWTGAVDQWGYGHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERRMENLNKKVDGFLDIWYNAELLVLLNERETLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFFFYHKCNNECMESVKNGTYDYPK YSEESKINREKIDGSESQVRQOFSKDIIEKLLNEQVKNEMQSSNLYMCSHNSYTHSLDAGGLETFDHAABEYEHAKKLLIFLNENNVFVQLTISIAPHEKKEGLTQIEOKAYEHEQHLSESINNLY DHAISKDHATENELQWYVAEQHEBEVLEFKOLLDKIELIGNENHGLVADQXVKGIASRKS	5
SIB 6247: HA(NC99 Y98F)bpFerr N19Q/C31S/S72C	<u>MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLL</u>EDSHNGKLCCLKGIAPIQLGNCVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHVTGVSASCSHNGKSSFYRNLLMTGKNGLYPNLSKSYVNKKEKVLVLMGVHPPNIGNORALYHTENAYSVVSSHYSRRFTPEIAKRPKVDRDQGRINYTYWTLLP GDTIIFEANGNLIAFWYAFALSRFGSGIITSNAPMDECDACQTPQGAINSLSLFFQNVHPTIGECPKYRSAKLRMTGLRNIPORETRGLFGAAGFTIEGGWTGAVDQWGYGHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERRMENLNKKVDGFLDIWYNAELLVLLNERETLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFFFYHKCNNECMESVKNGTYDYPK YSEESKINREKIDGSESQVRQOFSKDIIEKLLNEQVKNEMQSSNLYMCSHNSYTHSLDAGGLETFDHAABEYEHAKKLLIFLNENNVFVQLTISIAPHEKKEGLTQIEOKAYEHEQHLSESINNLY DHAISKDHATENELQWYVAEQHEBEVLEFKOLLDKIELIGNENHGLVADQXVKGIASRKS	6
SIB 6248: HA(NC99 Y98F)bpFerr	<u>MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLL</u>EDSHNGKLCCLKGIAPIQLGNCVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHVTGVSASCSHNGKSSFYRNLLMTGKNGLYPNLSKSYVNKKEKVLVLMGVHPPNIGNORALYHTENAYSVVSSHYSRRFTPEIAKRPKVDRDQGRINYTYWTLLP GDTIIFEANGNLIAFWYAFALSRFGSGIITSNAPMDECDACQTPQGAINSLSLFFQNVHPTIGECPKYRSAKLRMTGLRNIPORETRGLFGAAGFTIEGGWTGAVDQWGYGHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERRMENLNKKVDGFLDIWYNAELLVLLNERETLDFHDSNVKNLYEKVKSQLKNNAKEIGNGCFFFYHKCNNECMESVKNGTYDYPK YSEESKINREKIDGSESQVRQOFSKDIIEKLLNEQVKNEMQSSNLYMCSHNSYTHSLDAGGLETFDHAABEYEHAKKLLIFLNENNVFVQLTISIAPHEKKEGLTQIEOKAYEHEQHLSESINNLY DHAISKDHATENELQWYVAEQHEBEVLEFKOLLDKIELIGNENHGLVADQXVKGIASRKS	7

N19Q/C31S/A75C	GDTIIFEANGNLIAFWYAFALSRFGSGIIITSNAPMDECDACQTPQAGINSLPQNVHPVTIGCEPKYRSAKLRMTGLRNIPORETRGLFGAIAAGTIEGGWTGAVDQWGYHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERMENLNKKVDDGFLDIWYNALVLLLENERTLFDHSDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV YSEESKIANREKIDENLYEQGSESOVRQOFSKDIIEKLLNEQVKNEMOSSNLYMSHSSMSYTHSLDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV DHAIAKSKDHATENELQWVVAEQHEBEVLFEKDIIDKIELIGNENHGLYLADQVXKGIKAKSRKS
SIB 6249; HA(NC99 Y98F)bpFerr N19Q/C31S/S100C	MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLSHNGKLCCLKGIAPLQAGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHTVTGVSASCSHNGKSSFYRNLLMLTGKNGLYPNLSKSYVNKEKEVLVLMGVHPPNIGNORALYHTENAVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYTYWTLLP GDTIIFEANGNLIAFWYAFALSRFGSGIIITSNAPMDECDACQTPQAGINSLPQNVHPVTIGCEPKYRSAKLRMTGLRNIPORETRGLFGAIAAGTIEGGWTGAVDQWGYHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERMENLNKKVDDGFLDIWYNALVLLLENERTLFDHSDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV YSEESKIANREKIDENLYEQGSESOVRQOFSKDIIEKLLNEQVKNEMOSSNLYMSHSSMSYTHSLDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV DHAIAKSKDHATENELQWVVAEQHEBEVLFEKDIIDKIELIGNENHGLYLADQVXKGIKAKSRKS
SIB 6250; HA(NC99 Y98F)bpFerr N19Q/C31S TEV- S111C	MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLSHNGKLCCLKGIAPLQAGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHTVTGVSASCSHNGKSSFYRNLLMLTGKNGLYPNLSKSYVNKEKEVLVLMGVHPPNIGNORALYHTENAVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYTYWTLLP GDTIIFEANGNLIAFWYAFALSRFGSGIIITSNAPMDECDACQTPQAGINSLPQNVHPVTIGCEPKYRSAKLRMTGLRNIPORETRGLFGAIAAGTIEGGWTGAVDQWGYHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERMENLNKKVDDGFLDIWYNALVLLLENERTLFDHSDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV YSEESKIANREKIDENLYEQGSESOVRQOFSKDIIEKLLNEQVKNEMOSSNLYMSHSSMSYTHSLDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV DHAIAKSKDHATENELQWVVAEQHEBEVLFEKDIIDKIELIGNENHGLYLADQVXKGIKAKSRKS
SIB 6252; HA(NC99 Y98F)bpFerr N19Q/C31S TEV- S26C	MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLSHNGKLCCLKGIAPLQAGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHTVTGVSASCSHNGKSSFYRNLLMLTGKNGLYPNLSKSYVNKEKEVLVLMGVHPPNIGNORALYHTENAVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYTYWTLLP GDTIIFEANGNLIAFWYAFALSRFGSGIIITSNAPMDECDACQTPQAGINSLPQNVHPVTIGCEPKYRSAKLRMTGLRNIPORETRGLFGAIAAGTIEGGWTGAVDQWGYHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERMENLNKKVDDGFLDIWYNALVLLLENERTLFDHSDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV YSEESKIANREKIDENLYEQGSESOVRQOFSKDIIEKLLNEQVKNEMOSSNLYMSHSSMSYTHSLDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV DHAIAKSKDHATENELQWVVAEQHEBEVLFEKDIIDKIELIGNENHGLYLADQVXKGIKAKSRKS
SIB 6253; HA(NC99 Y98F)bpFerr N19Q/C31S TEV- S72C	MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLSHNGKLCCLKGIAPLQAGNCSVAGWILGNPECELLISKESWSYIVETPNPENGTCFPGYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHTVTGVSASCSHNGKSSFYRNLLMLTGKNGLYPNLSKSYVNKEKEVLVLMGVHPPNIGNORALYHTENAVSVVSSHYSRRFTPEIAKRPKVRDQEGRINYTYWTLLP GDTIIFEANGNLIAFWYAFALSRFGSGIIITSNAPMDECDACQTPQAGINSLPQNVHPVTIGCEPKYRSAKLRMTGLRNIPORETRGLFGAIAAGTIEGGWTGAVDQWGYHHQNEQGSYA ADQKSTQNAINGITNKVNSVIERKMTQTAVGKEFNKLERMENLNKKVDDGFLDIWYNALVLLLENERTLFDHSDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV YSEESKIANREKIDENLYEQGSESOVRQOFSKDIIEKLLNEQVKNEMOSSNLYMSHSSMSYTHSLDAGLETFDHAABEVEHAKKLLIENENNVFVQLTISIACEHKFEGLTQIFOKAYEHEQHISEINNIV DHAIAKSKDHATENELQWVVAEQHEBEVLFEKDIIDKIELIGNENHGLYLADQVXKGIKAKSRKS

SIB 6254; HA(NC99-Y98F)bfp^{ferr} N19Q/C31S TEV-A75C	YSESKIANREKID^{EMLYEQGSE}SQVRQOF^{SKDI}EKLANEQVNKEMOSSNLYMSMSSWYTHSLDGAGLEFEDHAAEEYEHAKKLIIFUNNNV^{FVOLT}CI^{SAPBHKFEGL}TOI^{EOKAYEHEQHISE} SINNIVDHAIKSKOHATEN^{LOWVVAEQHEEVEVL}FKDILDKIELIGNENHGILY^{ADQVVKGI}AKSRKS MKAKLLVLLCTFTATYADTICIGYHANNSTDVDTVLEKNVTVTSHVNLLED^{SHNGK}CLLKGIAPLQ^{LGNC}SVAGN^{ILGN}PECELLIS^{KESW}SYIVETPNPENGTC^{FP}GYFADYEBELREQLSSVS SFERPEIFPKTSSWPNH^{VT}GV^{SASCSHNGKSS}FYRNLLMLT^{GRNGLY}PNLSKSYVN^{NKE}REVLV^{MGVHP}PNIGNORALYHTENAYVSVVSHYSRRFTPEIAKRPKVRDQ^{EGR}INYYTWLTLEP GDTIIFEANGNLIAFWYAFALSRGFGSGIIITSNAPMEDCAKQTPQGA^{INS}SLPFQNVHPTTIGECPKYVRS^{AKLR}MTGLRNIPORETRGLFGA^{LAGT}IEGGWTGAVD^{GWYGYHHQNEQ}SGSYA ADQKSTQNAINGITNKVNSVIERKMTQFTAVGKEFNKLERMENLNK^{KVDDG}FLDIW^{TYN}AE^{LLV}LENER^{TLD}FHD^{SNVKNLY}EKVK^{SQ}LK^{NNAKE}IGNGCFFYHKCNNEC^{ME}SVKNGTYDYPK YSESKIANREKID^{EMLYEQGSE}SQVRQOF^{SKDI}EKLANEQVNKEMOSSNLYMSMSSWYTHSLDGAGLEFEDHAAEEYEHAKKLIIFUNNNV^{FVOLT}SI^{CEPHKFEGL}TOI^{EOKAYEHEQHISE} SINNIVDHAIKSKOHATEN^{LOWVVAEQHEEVEVL}FKDILDKIELIGNENHGILY^{ADQVVKGI}AKSRKS	12
SIB 6255; HA(NC99-Y98F)TEV bfp^{ferr}-N19Q/C31S/S100C	MKAKLLVLLCTFTATYADTICIGYHANNSTDVDTVLEKNVTVTSHVNLLED^{SHNGK}CLLKGIAPLQ^{LGNC}SVAGN^{ILGN}PECELLIS^{KESW}SYIVETPNPENGTC^{FP}GYFADYEBELREQLSSVS SFERPEIFPKTSSWPNH^{VT}GV^{SASCSHNGKSS}FYRNLLMLT^{GRNGLY}PNLSKSYVN^{NKE}REVLV^{MGVHP}PNIGNORALYHTENAYVSVVSHYSRRFTPEIAKRPKVRDQ^{EGR}INYYTWLTLEP GDTIIFEANGNLIAFWYAFALSRGFGSGIIITSNAPMEDCAKQTPQGA^{INS}SLPFQNVHPTTIGECPKYVRS^{AKLR}MTGLRNIPORETRGLFGA^{LAGT}IEGGWTGAVD^{GWYGYHHQNEQ}SGSYA ADQKSTQNAINGITNKVNSVIERKMTQFTAVGKEFNKLERMENLNK^{KVDDG}FLDIW^{TYN}AE^{LLV}LENER^{TLD}FHD^{SNVKNLY}EKVK^{SQ}LK^{NNAKE}IGNGCFFYHKCNNEC^{ME}SVKNGTYDYPK YSESKIANREKID^{EMLYEQGSE}SQVRQOF^{SKDI}EKLANEQVNKEMOSSNLYMSMSSWYTHSLDGAGLEFEDHAAEEYEHAKKLIIFUNNNV^{FVOLT}SI^{SAPBHKFEGL}TOI^{EOKAYEHEQHISE} CINNIVDHAIKSKOHATEN^{LOWVVAEQHEEVEVL}FKDILDKIELIGNENHGILY^{ADQVVKGI}AKSRKS	13
SIB9063; HA(南开-罗荣炳州 1918, Y98F)bfp^{ferr} N19Q/C31S/S111C	MEARLLVLLCA^{FAT}ATNADTICIGYHANNSTDVDTVLEKNVTVTSHVNLLED^{SHNGK}CLLKGIAPLQ^{LGNC}NTAGML^{LGNC}PECDLLLTASSWSYIVETSNSENGTC^{FP}GD^{FID}YEBELREQLSSVS SFERPEIFPKTSSWPNH^{ET}TKGV^{TAA}CSYAGASSFYRNLLMLT^{TKGSS}YPKLSKSYVN^{NKE}REVLV^{MGVHP}PTGTDQ^{QSLYQ}NADAYSVGSSKYNRRFTPEIAARPKVRDQ^{AGR}MNTYTWLTLE PGDTITFEATGNLIAFWYAFALNRGSGSGIIITSDAPVHDC^{NT}KQTPHGA^{INS}SLPFQNIHPVTIGECPKYVRS^{TKLR}MTGLRNI^{PSI}QSRGLFGA^{LAGT}IEGGWTGAVD^{GWYGYHHQNEQ}SGSGY AADQKSTQNAIDGITNKVNSVIERKMTQFTAVGKEFNLERRIENLNK^{KVDDG}FLDIW^{TYN}AE^{LLV}LENER^{TLD}FHD^{SNVKNLY}EKVK^{SQ}LK^{NNAKE}IGNGCFFYHKCNNEC^{ME}SVKNGTYDYP KYSEESKLNREEID^{SGGE}SQVRQOF^{SKDI}EKLANEQVNKEMOSSNLYMSMSSWYTHSLDGAGLEFEDHAAEEYEHAKKLIIFUNNNV^{FVOLT}SI^{SAPBHKFEGL}TOI^{EOKAYEHEQHISE} IYDHAIKCQKHATEN^{LOWVVAEQHEEVEVL}FKDILDKIELIGNENHGILY^{ADQVVKGI}AKSRKS	14
SIB9065; HA(加利福尼亚 2009, Y98F)bfp^{ferr} N19Q/C31S/S111C	MKAILVLLLYT^{FAT}ANADTICIGYHANNSTDVDTVLEKNVTVTSHVNLLED^{SHNGK}CLLKRGVAP^{HLGNC}NTAGML^{LGNC}PECELS^{TASSWSYIVET}TPSSDNGTC^{FP}GD^{FID}YEBELREQLSSVS SFERPEIFPKTSSWPNHNDSNKG^{VTAA}CPHAGAKSFYK^{NLIWLV}TKGNSY^{PKLSKY}INDK^{QEV}LVLMG^{IHH}PTSDAQ^{QSLYQ}NADTYVFGSSRYK^{PKF}PEIAIRPKVRDQ^{EGR}MNTYTWLTLE PGDKITFEATGNLVVPRYAFAMERNAGSGIIITSD^{FPVHDC}NTCQTPKGA^{INT}SLPFQNIHPITIGKCPKYVRS^{TKLR}MTGLRNI^{PSI}QSRGLFGA^{LAGT}IEGGWTGAVD^{GWYGYHHQNEQ}SGSGY AADLKSQNAIDIEITNKVNSVIERKMTQFTAVGKEFNLERRIENLNK^{KVDDG}FLDIW^{TYN}AE^{LLV}LENER^{TLD}FHD^{SNVKNLY}EKVK^{SQ}LK^{NNAKE}IGNGCFFYHKCNNEC^{ME}SVKNGTYDYP KYSEBAKLNREEID^{SGGE}SQVRQOF^{SKDI}EKLANEQVNKEMOSSNLYMSMSSWYTHSLDGAGLEFEDHAAEEYEHAKKLIIFUNNNV^{FVOLT}SI^{SAPBHKFEGL}TOI^{EOKAYEHEQHISE} IYDHAIKCQKHATEN^{LOWVVAEQHEEVEVL}FKDILDKIELIGNENHGILY^{ADQVVKGI}AKSRKS	15

SIB9066: HA(马来西 亚 1954 , Y98F)bpFerr N19Q/C3IS/S111C	16	<u>MCARLLIILLCAL</u>SATDADTICIGYHANNSTDVDTVLEKNVTVTHSVNLLSDSHNGKLCRLKGIAPQLQKCNIAWILGNPECESLLSNRSWSYIAETPNSENGICFPDGFADYEBELREQLSSVS SFERPEIFPKERSWPKHNITRGVTACSHAGKS SFYKNLLWLTEANGLYPSLSKSYVNDREKEVLVLWGHHPSNIEDQRTLYKENAYVSVSSNYNRRFTPEIAERPKVYRGQAGRINYTWLLE PGDKIIFRANGNLIAPWYAFALSRDGFSGGIITSNASMDCCD TKCQT PQGAINSLPFOQNIHPVTIGECPKYVRSTKLRMVTGLRNI PS IQSRGLFGA IAGFIEGGWTGMIDGWYGYHHQNEQSGSY AADQKSTQNAINGITNKVNSVIERKNTQFTAVGKEFNKLEKRMENLANKVDGDFLD IWTYNALLVLENERITLDFHDSNVKNLYEKYNQLRNNAKEIIGNGCFFEFYHKCNCECME SVKNGTYDYP KYSESKLNREKID <u>SGGESQVRQOFSKDI</u>EKLLNEQVKNEMOSSNLYMSMSWSYTHSDGAGLEFDHAAEEYEHAKKLLIIFLNENNVPVOLTISIAPERHKEEGLTOIFOKAYEHEQHSI SESINN IVDHAIKCKDHATENFLQWYVAEQHEEEVLEFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS
SIB9067: HA(蒙 斯 堡 1947 , Y98F)bpFerr N19Q/C3IS/S111C	17	<u>MCARLLIILLCAL</u>TATDADTICIGYHANNSTDVDTVLEKNVTVTHSVNLLSDSHNGKLCRLKGIAPQLQKCNIAWILGNPECESLLSKSWSYIAETPNSENGACFPDGFADYEBELREQLSSVS SFERPEIFPKERSWPKHNITRGVTAACSHAGKS SFYKNLLWLTE DGSY PKLSKSYVNKEKEVLVLWGHHPSNIEDQRTLYKENAYVSVSSNYNRRFTPEIAERPKVYRGQAGRINYTWLLE PGDTIIFRANGNLIAPWYAFALSRDGFSGGIITSNASMDCCD TKCQT PQGAINSLPFOQNIHPVTIGECPKYVRSTKLRMVTGLRNI PS IQSRGLFGA IAGFIEGGWTGMIDGWYGYHHQNEQSGSY AADQKSTQNAINGITNKVNSVIERKNTQFTAVGKEFNKLEKRMENLANKVDGDFLD IWTYNALLVLENERITLDFHDSNVKNLYEKYNQLRNNAKEIIGNGCFFEFYHKCNCECME SVKNGTYDYP KYSESKLNREKID <u>SGGESQVRQOFSKDI</u>EKLLNEQVKNEMOSSNLYMSMSWSYTHSDGAGLEFDHAAEEYEHAKKLLIIFLNENNVPVOLTISIAPERHKEEGLTOIFOKAYEHEQHSI SESINN IVDHAIKCKDHATENFLQWYVAEQHEEEVLEFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS
SIB9068: HA(香港 1977, Y98F)bpFerr N19Q/C3IS/S111C	18	<u>MCARLLIILLCAL</u>SATDADTICIGYHANNSTDVDTVLEKNVTVTHSVNLLSDSHNGKLCRLKGIAPQLQKCNIAWILGNPECESLFSKKSWSYIAETPNSENGTCFPDGFADYEBELREQLSSVS SFERPEIFPKERSWPKHNITRGVTASC SHGKS SFYRNLLWLTEKNGSYPNLSKSYVNKEKEVLVLWGHHPSNIEDQRTLYKENAYVSVSSNYNRRFTPEIAERPKVYRGQAGRINYTWLLE PGDTIIFRANGNLIAPWYAFALSRDGFSGGIITSNASMDCCD TKCQT PQGAINSLPFOQNIHPVTIGECPKYVRSTKLRMVTGLRNI PS IQSRGLFGA IAGFIEGGWTGMIDGWYGYHHQNEQSGSY AADQKSTQNAINGITNKVNSVIERKNTQFTAVGKEFNKLEKRMENLANKVDGDFLD IWTYNALLVLENERITLDFHDSNVKNLYEKYSQLKNNAKEIIGNGCFFEFYHKCNCECME SVKNGTYDYP KYSESKLNREKID <u>SGGESQVRQOFSKDI</u>EKLLNEQVKNEMOSSNLYMSMSWSYTHSDGAGLEFDHAAEEYEHAKKLLIIFLNENNVPVOLTISIAPERHKEEGLTOIFOKAYEHEQHSI SESINN IVDHAIKCKDHATENFLQWYVAEQHEEEVLEFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS
SIB9070: HA(H2, 1957, Y98F)bpFerr N19Q/C3IS/S111C	19	<u>MAIIYIILLLFTAVRCDQICIGYHANNSTEKVDITILERNVTVTHAKD ILEKTHNGKLCKLNGI PPLEIGDCS</u>IAGWLLGNPECDRLLSVPEWSYIMEKENPPDGLCFPGSPNDYEBELKHLSSVKHF ERVKILPKDRWTQHTTTGGRACAVSGNPSFRNNAWLTKESSNYPVAKGSYNNNTSGEQLI IWGVHPNDETEQRTLYQNVGTYSVGTSTLNKRSTPD IATRPKNVGLSRMEFSWTLLDMMDT INFESTGNLIAPEYGFKISKRGSSGIMTEGTLENCE TKCQT PLGAIN TILPFHNVHPLTIGECPKYVSEKVLATGLRNPQIESRGLFGA IAGFIEGGWQGMVDGWYGYHHSNDQSGSYAADK ESTQKAFDGI TNKNSVIERKNTQFTAVGKEFNKLEKRMENLANKVDGDFLD IWTYNALLVLENERITLDFHDSNVKNLYEKYNQLRNNAKEIIGNGCFFEFYHKCNCECME SVKNGTYDYP KYTEE ESKLNREBIKSGGESQVRQOFSKDI EKLNEQVKNEMOSSNLYMSMSWSYTHSDGAGLEFDHAAEEYEHAKKLLIIFLNENNVPVOLTISIAPERHKEEGLTOIFOKAYEHEQHSI SESINN IVDH ALKCKDHATENFLQWYVAEQHEEEVLEFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS
SIB9072 : HA(HSN1, 印度尼西	20	<u>MEKIVILLAIIVSLVKS</u>DQICIGYHANNSTEQVDTIMEKNVTVTHAQD ILEKTHNGKLCDLQGVKPLILRDCSVAGWLLGNPMKDEFINVPEWSYIVEKANPTNDLCFPGPSNDYEBELKHLSSRINH FEKIQIIPKSSWSDHEASGVS SACPYIGSPSFFFNVNLTKKNSYTPYTIKKS YNNNTNQEDLLVINGIHHNDAAEQFRLYQNPFTTYSIGTSTINQRLVPKIATRSKVNQSGSRMEFFWTILKPN

亚 2005, Y98F, 单 碱基的 bfpFerr N19Q/C31S/S11C	DAINFESNGNFIAPYAYKIYKGD ^{SAIMKSELEYGNCNTRCQT} PMGAINGSMPPHN ^{IHLPTIGCEPKYKSNRLVATGLRNS} <u>FOR</u> TRGLFGA ^{AGFTEGGWQGMVDGWYGYHHSNEQSGGYAA} DKESTQKALDGV ^{TNKNVSIIDKMTQTFEAVGREFNFLERRIENLNKKNEDGFLDVTWYNALLVLMENERITLDFHDSNVKNLYDKVRLQLRDNKAKELNGCGFEEYHKCDNECMESIRNGTYNYPQY} SEEARLKREETS ^{SGGESQVRQOF} SKDIEKLLNEQV ^{NKEMOSNLVMSMSWSYTHSLDGAGLEL} FDHAAEEYEHAKKLLI ^{FIENNNVYQVLTISI} SAPEHKEFGL ^{TOIFOKAYEHEQHI} SESINNLY DHAIKCKDHATENELQWVVAEQHEEVL ^{EFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS}	21
SIB9007: HA(丹佛 1957, Y98F)bfpFerr N19Q/C31S/S11C	MKAKLLIILLCALSATD ^{ADTICIGYHANNSTDVDTVLEKNVTVTHSVNLLBDSHNGKICRLKGKAPQLQGNCTIAGWVLGNPECESLLSNRSWYIAETPNSENGTCFPGDFADYEBELREQLSSVS} SPFEREIPPKERSWPNHTTRGVTAACPHARKSS ^{FYKNLVMLTEANGSYPNLSRSYVNNQKEVVLVGVHPSNIEEORALYRKDNNAVSVSVSNYNRRFTPEIAKRPKYVDQSGRNYYTWLLEP} GDTIIFEATGNLIAFWYAFALSRGPGSGII ^{ITSNAPLDECDTKCQTFQGAINSLPFQNIHPTIGCECPKYVRSKTLRMVTLGRNIPVQSRGLFGAAGFTEGGWTCAMDGWYGYHHQNEQSGGYA} ADQKSTQNAINGITNKNVSVTEKMTQ ^{TAVGKEFNKLEKRMENLNKKVDDGFM} DWNTYNAELLV ^{LENERITLDFHDSNVKNLYEKVKQNQLRNNAKELNGCGFEFYHKCDNECMESVKNGTYDYPK} YSEESKIANREKID ^{SGGESQVRQOF} SKDIEKLLNEQV ^{NKEMOSNLVMSMSWSYTHSLDGAGLEL} FDHAAEEYEHAKKLLI ^{FIENNNVYQVLTISI} SAPEHKEFGL ^{TOIFOKAYEHEQHI} SESINNLY VDHAIKCKDHATENELQWVVAEQHEEVL ^{EFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS}	22
SIB9109 : HA(H3N8, 马 俄莫 俄州 2003 , Y98F)bfpFerr N19Q/C31S/S11C	MKTTIILILLTHWAYSQNPISGNNTAT ^{CLGHHAVANGTLVKTI} SDDQIEV ^{TNATELVQSI} SMGKICNNSY ^{RIIDGRNCTLIDAMLGD} PHCDAFQYEN ^{NWDLFIERSSAFNSC} FPYDIPDYASLRSI VASSGTL ^{EFTAE} GFTWTGVTQNGRSGACKRG ^{SAD} SFFSRLN ^{WLTKSGSYPTLVNTPNNKFNPKLYI} WGIHHPSSNQ ^{QTKLYIQESGRVTVTKRSQQTII} PNIGSRPPWVRGQSGRIS ^{IYWTIV} KPGDILMINSNGNLVAPRGYFKLTKGKSSV ^{ARS} DVPIDICVSECIT ^{PNGSISNDKPPQNVNPKYIGKCPKVI} RQNTLKLATG ^{MRNVPEKQIRGIFGAIAGFIENGWEGMVDGWYGFYQNSEGTGQ} AADLKSTQAAIDQINGKLN ^{RVIERTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELIVALENQH} TIDLTDAMNKL ^{FERTRQLRRENAEDMGGCGCFKIYHKCDNACIGSIRNGTYDHY} IYDEALNNR ^{FKIDSGGESQVRQOF} SKDIEKLLNEQV ^{NKEMOSNLVMSMSWSYTHSLDGAGLEL} FDHAAEEYEHAKKLLI ^{FIENNNVYQVLTISI} SAPEHKEFGL ^{TOIFOKAYEHEQHI} SESINNLY IYDHAIKCKDHATENELQWVVAEQHEEVL ^{EFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS}	23
SIB9110: HA(H3N8, 马 巴 里 2005 , Y98F)bfpFerr N19Q/C31S/S11C	MKTTIILILLTHWAYSQNPISGNNTAT ^{CLGHHAVANGTLVKTI} SDDQIEV ^{TNATELVQSI} SMGKICNNSY ^{RIIDGRNCTLIDAMLGD} PHCDVQYEN ^{NWDLFIERSSAFNSC} FPYDIPDYASLRSI STIVASGTL ^{EFTAE} GFTWTGVTQNGRSGACKRG ^{SAD} SFFSRLN ^{WLTKSGSYPTLVNTPNNKFNPKLYI} WGIHHPSSNQ ^{QTKLYIQESGRVTVTKRSQQTII} PNIGSRPPWVRGQSGRIS ^{IYWTIV} IVKPGDILMINSNGNLVAPRGYFKLTKGKSSV ^{ARS} DVPIDICVSECIT ^{PNGSISNDKPPQNVNPKYIGKCPKVI} RQNTLKLATG ^{MRNVPEKQIRGIFGAIAGFIENGWEGMVDGWYGFYQNSEGT} GQAADLKSTQAAIDQINGKLN ^{RVIERTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELIVALENQH} TIDLTDAMNKL ^{FERTRQLRRENAEDMGGCGCFKIYHKCDNACIGSIRNGTYD} HYIYRDEALNNR ^{FKIDSGGESQVRQOF} SKDIEKLLNEQV ^{NKEMOSNLVMSMSWSYTHSLDGAGLEL} FDHAAEEYEHAKKLLI ^{FIENNNVYQVLTISI} SAPEHKEFGL ^{TOIFOKAYEHEQHI} SESINI NNLYDHAIKCKDHATENELQWVVAEQHEEVL ^{EFKDILDKIELIGNENHGLYLADQYVKGIAKSRKS}	24
SIB9111: HA(H3N8, 马 阿博园 2003 , Y98F)bfpFerr N19Q/C31S/S11C	MKTTIILILLTHWAYSQNPISGNNTAT ^{CLGHHAVANGTLVKTI} TDQDIEV ^{TNATELVQSI} SMGKICNNSY ^{RVLDGRNCTLIDAMLGD} PHCDDFQYES ^{MDLFIERSASSNSC} FPYDIPDYASLRSI VASSGTL ^{EFTAE} GFTWTGVTQNGRSGACKRG ^{SAD} SFFSRLN ^{WLTKSGSYPTLVNTPNNKFNPKLYI} WGIHHPSSNQ ^{QTKLYIQESGRVTVTKRSQQTII} PNIGSRPPWVRGQSGRIS ^{IYWTIV} KPGDVLMINSNGNLVAPRGYFKLTKGKSSV ^{ARS} DALIDTCVSECIT ^{PNGSIPNDKPPQNVNPKYIGRCPKYI} RQNTLKLATG ^{MRNVPEKQIRGIFGAIAGFIENGWEGMVDGWYGFYQNSEGTGQ} AADLKSTQAAIDQINGKLN ^{RVIERTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNAELIVALENQH} TIDLTDAMNKL ^{FERTRQLRRENAEDMGGCGCFKIYHKCDNACIGSIRNGTYDHY}	

SIB9132: HA(B, 马萨诸塞州 2012, Y98F)bpFerr N19Q/C31S/S111C	IYRDEALANNRFXID <u>SGGE</u> SOVRRQOF SKDI EKLAEQVKNKEMOSSNLYMSMSWSYTHSIDGAGLEFEDHAAEYEHAKKLIIFLNENNVPVOLTISI SAPHKEFEGLTQI EOKAYEHQHI SESINN IYDHAIAKCQDHAATENELQRYVAEOHEEVEVLEFKDILDKIELLIGNENHGLYLAQOYVKGIAKSRKS
SIB9147: HA(B, 普吉岛 2013)bpFerr N19Q/C31S/S111C	25 MKAIIVLLMVTISNA <u>DRIC</u> TIGITSSNPHVVKATQGEVNVTVGIPIPLTTPTKSYFANKGTRGKLCPCINCLTDLDVALGRPMC VGTTPS AKASILHEVRPVTS GCFFIMHRTKIRQLANLL RGYENIRLSTQNVIDAERKAPGGPYRLGTSGSCP NATSKSGFATMAVAPKDNKNANPLTVVEPYICAGEBQIITVWGFHSDNKTKQMKLYGDSNPQKFTSSANGVTTHYVSQIIGFPDQTEDG GLPQSGRIVVDYMQPKGTGTIVYQGVLLPQKWCASGRSKVIKGSPLPIGEADCLHEYGGLNKS KPYITOKHAKAI GNCP IWKTP LKLANGTKYRPPAKLLKE ROFFGALAGFLEGGWEGM IAGWHGYTSHGAHGVAAADLKSTQEA INKTKNLSLELVKNLQRLSGAMDELHNEIILEIDKVDLDRADTIS SQIELAVLLSNEGI INSEDEHLLALERKLKMLGPSAVDI GNCGCFETKHK CNQTCIDRIAAGTFNAGEFSLP TFD SINI TA <u>SGGE</u> SOVRRQOF SKDI EKLAEQVKNKEMOSSNLYMSMSWSYTHSIDGAGLEFEDHAAEYEHAKKLIIFLNENNVPVOLTISI SAPHKEFEGLTQI EOKAYEHQHI SESINN IYDHAIAKCQDHAATENELQRYVAEOHEEVEVLEFKDILDKIELLIGNENHGLYLAQOYVKGIAKSRKS
SIB9158: HA(H1N1 COBRA-P1, Y98F)bpFerr N19Q/C31S/S111C	26 MKAIIVLLMVTISNA <u>DRIC</u> TIGITSSNPHVVKATQGEVNVTVGIPIPLTTPTKSYFANKGTRGKLCPCINCLTDLDVALGRPMC VGTTPS AKASILHEVRPVTS GCFFIMHRTKIRQLANLL RGYENIRLSTQNVIDAERKAPGGPYRLGTSGSCP NATSKSGFATMAVAPKDNKNANPLTVVEPYICAGEBQIITVWGFHSDNKTKQMKLYGDSNPQKFTSSANGVTTHYVSQIIGFPDQTEDG GLPQSGRIVVDYMQPKGTGTIVYQGVLLPQKWCASGRSKVIKGSPLPIGEADCLHEYGGLNKS KPYITOKHAKAI GNCP IWKTP LKLANGTKYRPPAKLLKE ROFFGALAGFLEGGWEGM IAGWHGYTSHGAHGVAAADLKSTQEA INKTKNLSLELVKNLQRLSGAMDELHNEIILEIDKVDLDRADTIS SQIELAVLLSNEGI INSEDEHLLALERKLKMLGPSAVDI GNCGCFETKHK CNQTCIDRIAAGTFNAGEFSLP TFD SINI TAAS <u>SGGE</u> SOVRRQOF SKDI EKLAEQVKNKEMOSSNLYMSMSWSYTHSIDGAGLEFEDHAAEYEHAKKLIIFLNENNVPVOLTISI SAPHKEFEGLTQI QI EOKAYEHQHI SESINN IYDHAIAKCQDHAATENELQRYVAEOHEEVEVLEFKDILDKIELLIGNENHGLYLAQOYVKGIAKSRKS
SIB9158: HA(H1N1 COBRA-P1, Y98F)bpFerr N19Q/C31S/S111C	27 MKARLLVLLCALAATD <u>ADTIC</u> IGYHANNSTDVDTVLEKNVTVTHSVNLLSHNGKLCRLKGIAPLQLGKCN TAGWLLGNPECESILSARSWSYIVETPNSENGTCFP GDFIDYEBELREQLSSVS SPFERFEIPFKESSWPNHNTTKGVTAAACSHAGKSSFYRNLLMLTKKGGSYPKLSKYVNKKEVLVLMGVHHPSTSDQQSLYQENAYVSVVSSNYNRRFTPEIAERPKVYRGQAGRANYYWTLLLE PGDTIIFEATGNLIAPWTAFALSRGSGSGIITSNASMHECNTKCQTPQGA INSSILPFQNIHPVTIGECPKYVRSTKLRMWTGLRNIPSIQSRGLFGALAGFIEGGWTGMIDGWYGYHHQNEQSGSGY AADQKSTQNAINGITNKVNSVIERANTOFTAVGKEFNLEKRMENLANKKVDGDFIDITWTYNAELLVLENERITLDFHDSNVKNLYEKVKSQLRNNAKEI GNCGCFEYHKKCDNECME SVKNGTYDYP KYSEBSKLNREKID <u>SGGE</u> SOVRRQOF SKDI EKLAEQVKNKEMOSSNLYMSMSWSYTHSIDGAGLEFEDHAAEYEHAKKLIIFLNENNVPVOLTISI SAPHKEFEGLTQI EOKAYEHQHI SESINN IYDHAIAKCQDHAATENELQRYVAEOHEEVEVLEFKDILDKIELLIGNENHGLYLAQOYVKGIAKSRKS
SIB9160: HA(H1N1 COBRA-X3, Y98F)bpFerr N19Q/C31S/S111C	28 MEARLLVLLCAFAATNAD <u>DTIC</u> IGYHANNSTDVDTVLEKNVTVTHSVNLLSHNGKLCRLKGIAPLQLGKCN TAGWLLGNPECESILSARSWSYIAETPNPENGTCFP GYFADYEBELREQLSSVS SPFERFEIPFKESSWPNHNTTKGVTAAACSHAGKSSFYRNLLMLTKKGGSYPKLSKYVNKKEVLVLMGVHHPSTSDQQSLYQENAYVSVVSSNYNRRFTPEIAERPKVYRGQAGRANYYWTLLLE PGDTIIFEATGNLIAPWTAFALSRGSGSGIITSNASMDECDKACQTPQGA INSSILPFQNIHPVTIGECPKYVRSTKLRMWTGLRNIPSIQSRGLFGALAGFIEGGWTGMIDGWYGYHHQNEQSGSGY AADQKSTQNAINGITNKVNSVIERANTOFTAVGKEFNLEKRMENLANKKVDGDFIDITWTYNAELLVLENERITLDFHDSNVKNLYEKVKSQLRNNAKEI GNCGCFEYHKKCDNECME SVKNGTYDYP KYSEBSKLNREKID <u>SGGE</u> SOVRRQOF SKDI EKLAEQVKNKEMOSSNLYMSMSWSYTHSIDGAGLEFEDHAAEYEHAKKLIIFLNENNVPVOLTISI SAPHKEFEGLTQI EOKAYEHQHI SESINN IYDHAIAKCQDHAATENELQRYVAEOHEEVEVLEFKDILDKIELLIGNENHGLYLAQOYVKGIAKSRKS

SIB9162; HA(H1N1 COBRA-X6 , Y98F)bfpFerr N19Q/C31S/S111C	MEARLLVLLCAFAATNADTICIGYHANNSTDTVDTVLEKNVTVTHSVNLLSDSHNGKLCILKGIAPLQIGNCVAGWILGNPECELLISKESWSYIVETPNPENGTCFPQGYFADYEELREQLSSVS SFEREIEFPKSSWFNHTVTGVASCSHNGKSSFFENLLMLTGKNGLYPNLSKS YANNKEEVLVLHGWHPPNIGDORALYHTEENAVSVVSVSHYSRKTPEIAKRPKVVDQGRINYYTWLTLEP GDTIIFEANGNLIAPRYAPALSRGFGSGIITSNAFMD ECAKQTPQAGINS LFPQNVHPTVIGECPKYRS AKLRMTGLRNIPSIQSRGLFGALAGFIEGGWTGAVD GWYGYHHQNEQSGSYA ADQKSTQNAINGITNKVNSVTEKMTQTAVGKEPNKLERMENLKKVDDGFID IWTYNABELLVLENERTLDFHDSNVKNLYEKVKSQ LKNNAKEIGNGCFFYHKCNNECHESVKNGTYDYPK YSESKIANREKIDSGGESQVRQOFSKDI EKLLNEQVKNEMOSSNLYMSMSWSYTHSLDGAGLEFDHAAEEVEHAKKLIIFINENNVPVOLTISI SAPEHKEEGLTOIFOKAYEHEQHI SESINN VDHAIKCKDHATENELQWYVAEQHEEVELEKOLLDKIELIIGNENHGLYLADQVVKGIASRKS	29
SIB9164 : HA(H3N2 , 伯斯 2009)bfpFerr N19Q/C31S/S111C	MKTIIALSYLCLVFAQKLPONDNSTALCIGHHVPNGTIVKTIITNDQIEVTNATELVQSSSTGEICDSPHQIILDGKNTLIDALLGD PQDQFQNKKWDLFVRSKAYSNCYPYDVPDYASLRS LVASSGTLFEFNESFNWTGVTQNGTSSACIRRSKNSFFSRLNWLTHLNFKYPALNVTMPNNEQDKLYINGVHHPGTDKQIFLYAQASGRITVSTKRSQQTVPSPNIGSRPRVRNIPSRISYIWTI VKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCNSECIITNGSIPNDKPFQNVNRIITYGACPRYVQNTLKLATGGRNVPEKQTRGIFGALAGFIEENGWEGMVDGWYGFHQNSEBORG QAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNABELLVALENQHTIDLTDSEANKLFKTKQLRENAEDMNGCFKIYHKCDNACIGSIRNGTYDH DVYRDEALNNRFQIKSGGESQVRQOFSKDI EKLLNEQVKNEMOSSNLYMSMSWSYTHSLDGAGLEFDHAAEEVEHAKKLIIFINENNVPVOLTISI SAPEHKEEGLTOIFOKAYEHEQHI SESIN NIVDHAIKCKDHATENELQWYVAEQHEEVELEKOLLDKIELIIGNENHGLYLADQVVKGIASRKS	30
SIB9167; HA(H3N2 维多利 亚 2011)bfpFerr N19Q/C31S/S111C	MKTIIALSYLCLVFAQKLPONDNSTALCIGHHVPNGTIVKTIITNDQIEVTNATELVQSSSTGEICDSPHQIILDGKNTLIDALLGD PQDQFQNKKWDLFVRSKAYSNCYPYDVPDYASLRS LVASSGTLFEFNESFNWTGVTQNGTSSACIRRSKNSFFSRLNWLTHLNFKYPALNVTMPNNEQDKLYINGVHHPTDKQIFLYAQSSGRITVSTKRSQOAVIPNIGYPRIRNIPSRISYIWTI VKPGDILLINSTGNLIAPRGYFKIRSGKSSIMRSDAPIGKCNSECIITNGSIPNDKPFQNVNRIITYGACPRYVQNTLKLATGGRNVPEKQTRGIFGALAGFIEENGWEGMVDGWYGFHQNSEBORG QAADLKSTQAAIDQINGKLNRLIGKTNEKFHQIEKEFSEVEGRIQDLEKYVEDTKIDLWSYNABELLVALENQHTIDLTDSEANKLFKTKQLRENAEDMNGCFKIYHKCDNACIGSIRNGTYDH DVYRDEALNNRFQIKSGGESQVRQOFSKDI EKLLNEQVKNEMOSSNLYMSMSWSYTHSLDGAGLEFDHAAEEVEHAKKLIIFINENNVPVOLTISI SAPEHKEEGLTOIFOKAYEHEQHI SESIN NIVDHAIKCKDHATENELQWYVAEQHEEVELEKOLLDKIELIIGNENHGLYLADQVVKGIASRKS	31
SIB9320 : HA(H5N1 , hCOBRA-2, Y98F, 单碱基的)bfpFerr N19Q/C31S/S111C	MEKIVILLALVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDDEFINVPWSYIVEKANPANDLCPFGNFNDYEELKHLLSRINH FEKIQIIPKSSWSDHEASSGVSSACPYQGSFSPFNVNLIKRNTYPTIKRSYNNTNQEDLLVLWGIHHNDAAEQTRLYQNPTTYSVTGSTINQRLVPKIA TRSKVNGQSGRM EFFFTILKPN DAINFESNGNFIAPYAYKIVKKGDSALMSELEYGNCKTKQTPIGAINSSMPFNHPLTI GECPKYKSNRLVATLGLRNS PQRETHGLFGALAGFIEGGWQGMVDGWYGYHHSNQSGSYAA DRESTQKALDGVTKNVNSIIDKNTQFPAVGRFBNLERLENLKNKQEDFLDVTWYNABELLVLENERTLDFHDSNVKMLYDKVRLQLDNNAKEIGNGCFFYHKCDNECHESVNGTYDYPQY SEEARLKREETS SGGESQVRQOFSKDI EKLLNEQVKNEMOSSNLYMSMSWSYTHSLDGAGLEFDHAAEEVEHAKKLIIFINENNVPVOLTISI SAPEHKEEGLTOIFOKAYEHEQHI SESINNIV DHAIKCKDHATENELQWYVAEQHEEVELEKOLLDKIELIIGNENHGLYLADQVVKGIASRKS	32
SIB9321 : HA(H5N1 , 班共摩	MEKIVILLALVSLVKSDQICIGYHANNSTEQVDTIMEKNVTVTHAQDILEKTHNGKLCDLDGVKPLILRDCSVAGWLLGNPMCDDEFINVPWSYIVEKANPANDLCPFGNFNDYEELKHLLSRINH FEKIQIIPKSSWSDHEASSGVSSACPYQGSFSPFNVNLIKRNTYPTIKRSYNNTNQEDLLVLWGIHHNDAAEQTRLYQNPTTYSVTGSTINQRLVPKIA TRSKVNGQSGRM EFFFTILKPN	33

2005, Y98F, 单碱基的)bpFerr N19Q/C31S/S111C	DAINFESGNFTAPENAYKI VKGDSTIMKSELYGNCNTRCQTPIGAINSMFPFNHPLTIGECPRYKSNRLVATGLRNS PQIBTRGLFAGIAGFIEGGWQGMVDGWYGYHHSNEQSGYAA DKESTQKALDGVTKVNSIIDKMTQFEAVGREFNLERIENLNKMGDFLDVWTYNABLLVMENERTLDFHD SNVKNLYDKVRLQLRDNNAKELGNGCFEYHRCDCNECMESVRNGTYDYPOY SEEARLKREEIS SGGESQVRQOQFSKDI EKLNEQNKEMQSNLYMSMSWSYTHSLDGAGLEFEDHAAEEYEHAKKLI IELNENNVVQVLTISI SAPEHKFEGTLQIFOKAYEHEQHLSESINNLY DHAIKCKDHATFENELQWYVAEQHEBEVLFKDILDKIELIGNENHGLYLADQXVKGI AKSRKS	34
SIB9329 : HA(H5N1, 大天鹅 2005, Y98F, 单碱基的)bpFerr N19Q/C31S/S111C	MEKIVLLLAIVSLVKSQDQICIGYHANNSTEQVDTIMEKNVTTHAQDILEKTHNGKLCDDGVKPLIILDCSVAGWLLGNPMCDDEFINVPWSYIVKEKANPANDLC FPGNPDYEEELKHLLSRINH FEKIQIIPKSSWSDHEASSGVSSACPYQGRSSFFRNVMWLIKDNAYPTIKRSYNTNTQEDLLVWGIHHPNDAAEQTRLYQNPTTYSVGTSTINQRLVPKIATRSKVNQSGRMEFFFTILKPN DAINFESGNFTAPENAYKI VKGDSTIMKSELYGNCNTRCQTPIGAINSMFPFNHPLTIGECPRYKSNRLVATGLRNS PQIBTRGLFAGIAGFIEGGWQGMVDGWYGYHHSNEQSGYAA DKESTQKALDGVTKVNSIIDKMTQFEAVGREFNLERIENLNKMGDFLDVWTYNABLLVMENERTLDFHD SNVKNLYDKVRLQLRDNNAKELGNGCFEYHRCDCNECMESVRNGTYDYPOY SEEARLKREEIS SGGESQVRQOQFSKDI EKLNEQNKEMQSNLYMSMSWSYTHSLDGAGLEFEDHAAEEYEHAKKLI IELNENNVVQVLTISI SAPEHKFEGTLQIFOKAYEHEQHLSESINNLY DHAIKCKDHATFENELQWYVAEQHEBEVLFKDILDKIELIGNENHGLYLADQXVKGI AKSRKS	35
SIB9330 : HA(H5N1, 野鸭/华 东 2003, Y98F, 单碱基的)bpFerr N19Q/C31S/S111C	MEKIVLLLAIVSLVKSQDQICIGYHANNSTEQVDTIMEKNVTTHAQDILEKTHNGKLCDDGVKPLIILDCSVAGWLLGNPMCDDEFINVPWSYIVKEKANPANDLC FPGNPDYEEELKHLLSRINH FEKIQIIPKSSWSDHEASSGVSSACPYQGRSSFFRNVMWLIKDNAYPTIKRSYNTNTQEDLLVWGIHHPNDAAEQTRLYQNPTTYSVGTSTINQRLVPKIATRSKVNQSGRMEFFFTILKPN DAINFESGNFTAPENAYKI VKGDSTIMKSELYGNCNTRCQTPIGAINSMFPFNHPLTIGECPRYKSNRLVATGLRNS PQIBTRGLFAGIAGFIEGGWQGMVDGWYGYHHSNEQSGYAA DKESTQKALDGVTKVNSIIDKMTQFEAVGREFNLERIENLNKMGDFLDVWTYNABLLVMENERTLDFHD SNVKNLYDKVRLQLRDNNAKELGNGCFEYHRCDCNECMESVRNGTYDYPOY SEEARLKREEIS SGGESQVRQOQFSKDI EKLNEQNKEMQSNLYMSMSWSYTHSLDGAGLEFEDHAAEEYEHAKKLI IELNENNVVQVLTISI SAPEHKFEGTLQIFOKAYEHEQHLSESINNLY DHAIKCKDHATFENELQWYVAEQHEBEVLFKDILDKIELIGNENHGLYLADQXVKGI AKSRKS	36
SIB9332: HA(B, 希 里新班 2008 , D197N)pFerr N19Q/C31S/S111C	MDTLLWLLWLLWPGSTCDRICTGITSNSPHVVKATQGEVNVTVGVIPLTTPTKSHFANLKGTETRGKLCPCLNCTDLVALGRPKCTGKIP SARVSI LHEVRPVTSGCFFIMHDRTKIRQLP NLLRGYEHIRLSTHNVINAEAPGGFYKIGTSGSCFNITNGGFFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSANGVTTHYYSQIGGFPNQ TEDGGLPQSGRIWVDYMWQSGKTGTITYQRGILLPQKVCASGRSKVIKGLPLIGEADCLHEKYGLNKSPPYTGHAKAIGNCPITWKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGG WEGMTAGWHGTYSHGAGVAADLKSTQEAINKITKNINLSLELVNQLRQLSGMDLHNEIILEDEKVDLDRADTISQIELAVLLSNEGIINSEDEHLALERLKKMLGFSAVEIGNGCFFE TKHKCNQCLDRIAAGTFDAGEFSLPTFDLSINITAASSRSSGGDIKLLNEQNKEMQSNLYMSMSWSYTHSLDGAGLEFEDHAAEEYEHAKKLI IELNENNVVQVLTISI SAPEHKFEGTLQIFOKAYEHEQHLSESINNLY EQKAYEHEQHLSESINNLYDHAIKCKDHATFENELQWYVAEQHEBEVLFKDILDKIELIGNENHGLYLADQXVKGI AKSRKS	37
SIB9334: HA(B, 普 吉岛 2013)pFerr N19Q/C31S/S111C	MKAIIVLLWMTVSNADRICTGITSNSPHVVKATQGEVNVTVGVIPLTTPTKSHFANLKGTETRGKLCPCLNCTDLVALGRPMCVGTTSPAKASILHEVRPVTSGCFFIMHDRTKIRQLP RGYEKIRLSTQNVDAEKAPGGFYRLGSGCPNATSKIGFATMAWAVPKNDKNKTATNPLTIEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQKFTSSANGVTTHYYSQIGGFPDQEDG GLPQSGRIWVDYMWQSGKTGTITYQRGILLPQKVCASGRSKVIKGLPLIGEADCLHEKYGLNKSPPYTGHAKAIGNCPITWKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGM TAGWHGTYSHGAGVAADLKSTQEAINKITKNINLSLELVNQLRQLSGMDLHNEIILEDEKVDLDRADTISQIELAVLLSNEGIINSEDEHLALERLKKMLGFSAYDIGNGCFFETHKH	

	CNQTCIDRIAAGTFNAGEFSLPFTDSINI^{TAAS}SGGDIILKLLNEQVNMOSNNLMSMSWSYTHSDGAGLEFDHAAEYEHAKKLIIFLNENNVPVOLTISI^{SAP}EHKEFEGLTQIFOKAYEHE QHISESINNIVDHAIKCQDHATENELQWVAEQHEEVELEFKDILDKIELIGNENHGLYLADQVVKGI^{AKSRKS}	
SIB9337: HA(B, 希 里斯班 2008, D197N) ^{pFerr} N19Q/C31S/S111C	MKAIIVLLMVVTSN^{ADRI}CTGITSNSPHVVKATQGEVNVTVGIPLTTPTKSHFANLKGTRGKLCPCLNCTDLDVALGRPMCVGTTPPSAKASILHEVRPVTS^{GC}PFIMHRTKIRQLPNLL RGYEHIRLSTHRVINAENAPGGPYKIGTSGCPNITNGGFATMAVPRNDKNKTATNPLTTEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSKPQFTSSANGVTTHYVSQIGGFPNQTED GGLPQSGRIVVDYMQKSGKTGTITYQRIILLPQKWCASGRSKVIGSLPLIGEADCLHEKYGGLANKSPYTTGEHAKAIGNCPPIWKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEG MIAGWHGYTSHGAHGVAAADLKSTQEAINKITKNLNSILEVKNLQRLSGAMDELHNEILELDEKVDLDRADTIS^{SQ}IELAVLLSNEGIIINSEDEHLLALERKLLKMLGPSAVEIGNGCFETKH KCNQTCIDRIAAGTFNAGEFSLPFTDSINI^{TSGGDI}ILKLLNEQVNMOSNNLMSMSWSYTHSDGAGLEFDHAAEYEHAKKLIIFLNENNVPVOLTISI^{SAP}EHKEFEGLTQIFOKAYEHEQH ISESINNIVDHAIKCQDHATENELQWVAEQHEEVELEFKDILDKIELIGNENHGLYLADQVVKGI^{AKSRKS}	38
SIB9347: HA(B, 威 斯康星州 2010, Y98F) ^{pFerr} N19Q/C31S/S111C	MKAIIVLLMVVTSN^{ADRI}CTGITSNSPHVVKATQGEVNVTVGIPLTTPTKSHFANLKGTRGKLCPCLNCTDLDVALGRPMCVGTTPPSAKASILHEVRPVTS^{GC}PFIMHRTKIRQLPNLL RGYENIRLSTQNVIDA^{EKA}PGPYRLGTSGCPNATSKIGFFATMAVPRNDKNKTATNPLTTEVPYICTEGEDQITVWGFHSDNETQMAKLYGDSNPQFTSSANGVTTHYVSQIGDPPDQTEDG GLPQSGRIVVDYMQKPKGTGTIVYQRIILLPQKWCASGRSKVIGSLPLIGEADCLHEKYGGLANKSPYTTGEHAKAIGNCPPIWKTPLKLANGTKYRPPAKLLKERGFFGAIAGFLEGGWEGM IAGWHGYTSHGAHGVAAADLKSTQEAINKITKNLNSILEVKNLQRLSGAMDELHNEILELDEKVDLDRADTIS^{SQ}IELAVLLSNEGIIINSEDEHLLALERKLLKMLGPSAVDIGNGCFETKHK CNQTCIDRIAAGTFNAGEFSLPFTDSINI^{TSGGDI}ILKLLNEQVNMOSNNLMSMSWSYTHSDGAGLEFDHAAEYEHAKKLIIFLNENNVPVOLTISI^{SAP}EHKEFEGLTQIFOKAYEHEQH ISESINNIVDHAIKCQDHATENELQWVAEQHEEVELEFKDILDKIELIGNENHGLYLADQVVKGI^{AKSRKS}	39
SIB9379 : (H3- 基)bfp ^{Ferr} N19Q/C31S/S111C	MKTIIALSVILCLVFAQKLPNDNSTATLCLGHHAVPNGTVIKTITNDQIEVTNATELVFPQGVVLKATGRNVPEKQTRGIFGATAGFTIENGWEGMDVGYGFRHQNAGEGTAAADYKSTQSAIDQITGMV NQINGWNRVIELMEQGGPDCYLAELLVALLNQHVIDLTDSEMRKLFERTKKQLRENAEDMNGCFKIYHKCDNACIGSIRNGTYDHDVYRDEALNNRFQIKSGGESQVRQF^{SKDI}EKLLNEQVNMOSNN KEMOSSNLYMSN^{SS}SYTHSDGAGLEFDHAAEYEHAKKLIIFLNENNVPVOLTISI^{SAP}EHKEFEGLTQIFOKAYEHEQHISESINNIVDHAIKCQDHATENELQWVAEQHEEVELEFKDILDKI ELLIGNENHGLYLADQVVKGI^{AKSRKS}	40
SIB9380 : (H7- 基)bfp ^{Ferr} N19Q/C31S/S111C	MNTQILVFA^{LIAI}IPTNADKICLGHAVNSNGTKVNTLTERGVVVNATELVFPQGVVLKATGMNVPEIPKGRGLFGAIGFTIENGWEGMIDGWFYGRHQNAGEGTAAADYKSTQSAIDQITGMV NRVIELMEQGGPDCYLAELLVALLNQHVIDLADSEMDKLYERVVKQLRENAEDGTGCFE^{IFHK}CDDDCMASIRNNTYDHSKYREAMQNRIQIDSGGESQVRQF^{SKDI}EKLLNEQVNMOSNN LYMSN^{SS}SYTHSDGAGLEFDHAAEYEHAKKLIIFLNENNVPVOLTISI^{SAP}EHKEFEGLTQIFOKAYEHEQHISESINNIVDHAIKCQDHATENELQWVAEQHEEVELEFKDILDKIELIGNEN HGLYLADQVVKGI^{AKSRKS}	41
SIB9408 : (H10- 基)bfp ^{Ferr} N19Q/C31S/S111C	MYKIVVITIALLGAVKGLDKICLGHAVANGTIVKLTNEQEVTNATELVFPQGVVLKATGMNVPEIPKGRGLFGAIGFTIENGWEGMIDGWFYGRHQNAGEGTAAADYKSTQSAIDQITGMV RVVIELMEQGGPDCYLAELLVALLNQHVITMDADSEMN^{TY}YERVRKQLRENAEDGKCFE^{IFHAC}DDSCMESIRNNTYDHSKYREALLNRIN^{TS}SGGESQVRQF^{SKDI}EKLLNEQVNMOSNN LYMSN^{SS}SYTHSDGAGLEFDHAAEYEHAKKLIIFLNENNVPVOLTISI^{SAP}EHKEFEGLTQIFOKAYEHEQHISESINNIVDHAIKCQDHATENELQWVAEQHEEVELEFKDILDKIELIGNENH GLLYLADQVVKGI^{AKSRKS}	42

SIB9148 : (H1- 茎) bfpFerr N19Q/C31S/S111C	MKAKLLVLLCTFTATYADTICIGYHANNSTDTVDFTLEKNVTVTSHSVNLGSLRMVTLGIRNIPQRETRGLFAGTAGFTGGWTGMVDWGYGHHQNEQGSYAADQKSTQVAINGITNMYNSVIEK MGSQSGTDLAELLVLLNERTLDPHDNVRNLYEKVSQAKNAKEIGNGCFPFYHKORNECRESVKNGYDYPKTSSESKINREKIDSGESQVQOFSKQIEKLINEANKEKMOSSNLYMSMS SMSYTHSLDGAGLEFDHAAEEYEHAKKLIIFLNENNVFQLTISIAPHHKFEGLTQIFQKAYEHEQHISESINNIVDHAICKKDHATFNFLQWYVAEQHEEEVLFKDILOKIELIGNENHGLYLA DQYVKGIAKSRKS	43
TEV 切割位点	ENLYEQG	44
CpG (其中示出*的为现代 磷酸酯修饰)	T*G*A*C* ⁺ T*G* ⁺ T*G*A*A* ⁺ C*G* ⁺ T* ⁺ C*G* ⁺ A*G* ⁺ A* ⁺ T*G* ⁺ A	45
	未使用的	46-200
bfp 铁蛋白 - N19Q/C31S/S26C	ESQVPRQOFSKQIEKILLNEQVKNKEMQSSNLYMCMSSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVFQLTISIAPHHKFEGLTQIFQKAYEHEQHISESINNIVDHAICKKDHATFNFL QWYVAEQHEEEVLFKDILOKIELIGNENHGLYLDQYVKGIAKSRKS	201
bfp 铁蛋白 - N19Q/C31S/S72C	ESQVPRQOFSKQIEKILLNEQVKNKEMQSSNLYMSMSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVFQLTISIAPHHKFEGLTQIFQKAYEHEQHISESINNIVDHAICKKDHATFNFL QWYVAEQHEEEVLFKDILOKIELIGNENHGLYLDQYVKGIAKSRKS	202
bfp 铁蛋白 - N19Q/C31S/A75C	ESQVPRQOFSKQIEKILLNEQVKNKEMQSSNLYMSMSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVFQLTISCPHHKFEGLTQIFQKAYEHEQHISESINNIVDHAICKKDHATFNFL QWYVAEQHEEEVLFKDILOKIELIGNENHGLYLDQYVKGIAKSRKS	203
bfp 铁蛋白 - N19Q/C31S/K79C	ESQVPRQOFSKQIEKILLNEQVKNKEMQSSNLYMSMSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVFQLTISIAPHHKFEGLTQIFQKAYEHEQHISESINNIVDHAICKKDHATFNFL QWYVAEQHEEEVLFKDILOKIELIGNENHGLYLDQYVKGIAKSRKS	204
bfp 铁蛋白 - N19Q/C31S/S100C	ESQVPRQOFSKQIEKILLNEQVKNKEMQSSNLYMSMSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVFQLTISIAPHHKFEGLTQIFQKAYEHEQHISECINNIVDHAICKKDHATFNFL QWYVAEQHEEEVLFKDILOKIELIGNENHGLYLDQYVKGIAKSRKS	205
bfp 铁蛋白 - N19Q/C31S/S111C	ESQVPRQOFSKQIEKILLNEQVKNKEMQSSNLYMSMSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVFQLTISIAPHHKFEGLTQIFQKAYEHEQHISESINNIVDHAICKKDHATFNFL QWYVAEQHEEEVLFKDILOKIELIGNENHGLYLDQYVKGIAKSRKS	206
bfp 铁蛋白 - N19Q/C31S/E12C	ESQVPRQOFSKQIEKILLNEQVKNKEMQSSNLYMSMSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVFQLTISIAPHHKFEGLTQIFQKAYEHEQHISESINNIVDHAICKKDHATFNFL QWYVAEQHEEEVLFKDILOKIELIGNENHGLYLDQYVKGIAKSRKS	207
具有牛痘核头的示 例性曲门螺杆菌铁 蛋白	ESQVPRQOFSKQIEKILLNEQVKNKEMQSSNLYMSMSWSYTHSLDGAGLFLFDHAAEEYEHAKKLIIFLNENNVFQLTISIAPHHKFEGLTQIFQKAYEHEQHISESINNIVDHAICKKDHATFNFL QWYVAEQHEEEVLFKDILOKIELIGNENHGLYLDQYVKGIAKSRKS	208

示例野生型曲门 螺杆菌铁蛋白 (GenBank 登录 AAD06160.1) (没有 牛蛙接头或 N 末端 Met)	LSKDI IKLLNEQVNMKNSSNLYMSMSWCYTHSIDGAGLFDFHAAEEYEHAKKLIIFLNENNVVQLTTSISAFEHKFEGLTQIFQKAYEHEQHI SESINNIIVDHAIKSKDHATFNFLQWYVAEQ HEEEVLFKDIIDDKIELIGNENHGLYLADQYVKGIAKSRKS	209
CpG(SS-1018)	TGACTGTGAACGTTGAGATGA	210
粉纹夜蛾重链铁蛋白	TQCNVNPVQIPKDWITMHRSCRNMRQIQMEVGASLOYLAWGAHFSDVVRPGFAQLFFDAASEEREHAMKLIIEYLIMRGELTNDVSSLLQVRRPPTRSSWKGVEALEHLSMESDVTKSIINV IKACEDDSEFNDYHLVDYLTGDFLEEQYKGQDLAKGASTLKKLMRHEALGEF IFDKKLLGIDV	211
粉纹夜蛾轻链铁蛋白	ADTCYNDVALDCGITSNSLALPRCNNAVIGEYSGHGNVATELOAYAKLHLERSYDYLLSAAFYNNYQTNRAGESKLFKKLSDEAMSKTIDIIKHVTKRGDQNFQDQSTMTKTERKNYTAENHELEAL AKALDTQKELAEARAFYIHREATRNSQHLHDPEIAQYLEEEFIEDHAEKIRTLAGHTSDLKKFITANNHGLSLALYVFDEYLQKTV	212
激烈火球菌铁蛋白	MLSERMLKALNDQNLRELYSAYLYFAMAAYFEDLGLGFANWKAQAEEEEIGHALRFYNY IYDRNGRVELDEIPKPKWESEPLKAFAEAAVEHEKFI SKSIYELAALAEEEKDYSTRAFL EWFINEQVEEASVKKILDKLKEAKDSPQILEMLDKELSARAPKLPGLMQGGE	213
人重链铁蛋白	MTTASTSQVRQNYHQDSEAAINRQINLELYASYVYLSMSYTFDRDDVALKNFAKYFLHQSHEEREHAEKLMKLQNRGGRIFLQDIKKPCDDDWESGINAMECALHLEKNVQSSILLEHLKLATDKN DPHLCDFIETHYLINEQVKAIKELGDHVTNLRKMGAPESGLAEYLFDKHTLGDQES	214
人轻链铁蛋白(信号 肽以下划线表示)	<u>MDSKGSQKGSRLLLLVSNLLLPQGV</u> ASSQIRQNYSTDVEAAVNSLVNLYLQASYTILSGFYFDRDDVALEGVSHFFRELAEEKREGYERLLKMQNRQGRALFQDIKKPAEDEWGKTPDAM KAAVALEKKLNQALIDLHALGSARTDPHLCDFLETHFLDEEVKLIKMGDHLTNLHRLGGPEAGLGEYLFERLTIKHD	215
	未使用的	216
牛蛙接头	ESQVRQQF	217
牛脱氨酸-凝血酶- His 接头(牛脱氨酸以 双下划线表示)	<u>GLVPRGSLEHHHHH</u>	218
	未使用的	219
16 个氨基酸的接头	GGGGGGGGGGGGSG	220

28 个氨基酸的接头	GGSGSGSNSSASSGASGGASGGGGSG	221
46 个氨基酸的接头	GGSGSASSGASASGSSNGSGSGSGSNSSASSGASSGGASGGGGSG	222
FR1	GGSGSASAEAAAKEAAAKAGGGGGSG	223
FR2	GGSGSASAEAAAKEAAAKEAAKASGGGGSG	224
47 个氨基酸的接头， 其包含用于缀合的 C	SGGGSGSASSGASASGSCSGSGSGSSASSGASSGGASGGGGSG	225

实施例

[0231] 提供以下实施例以说明某些公开的实施方案，并且不应解释为以任何方式限制本

公开文本的范围。

1. 对HA-铁蛋白纳米颗粒的设计、纯化和表征

[0232] 通过以下方式产生HA纳米颗粒(HA-Np):将HA胞外结构域序列(缺乏48个C端跨膜残基)融合至铁蛋白的N末端以产生在哺乳动物细胞中自组装的纳米颗粒。铁蛋白包含用半胱氨酸置换表面暴露的氨基酸的突变(由相对于SEQ ID NO:208的铁蛋白序列的S26C、A75C或S111C突变产生),以允许将佐剂缀合至HA-Np上。这种半胱氨酸的表示呈现于图2中。

[0233] 由上文所述的突变产生的半胱氨酸可用于缀合免疫刺激部分。图3A说明了使用马来酰亚胺-PEG4-SM7/8a点击试剂将TLR激动剂缀合至铁蛋白的示例性1步式点击化学反应的结果。在此反应中,使马来酰亚胺与不成对半胱氨酸反应,以经由中间接头将TLR7/8激动剂分子在表面暴露的半胱氨酸处共价缀合至铁蛋白,从而产生共价的TLR7/8-激动剂-铁蛋白缀合物。在此例子中,指示了在一个单体的表面上的一个半胱氨酸。铁蛋白纳米颗粒是多聚体,例如由24个单体组成。因此,铁蛋白纳米颗粒可以包含等于单体数量的表面暴露的半胱氨酸数量,并且每个表面暴露的半胱氨酸可以被缀合。

[0234] 图3B和图4A至图4B说明了可以用于将佐剂缀合至铁蛋白纳米颗粒的示例性2步式点击化学,并且说明了CpG和3M-012(有时也称为3M012)也可经由中间双官能接头通过2步式点击化学反应缀合至铁蛋白的方式。示例性接头是含有马来酰亚胺和DBCO反应性基团的Sigma PEG接头(目录号760676)。在此例子中,TLR激动剂被反应性叠氮化物基团官能化。

[0235] 将质谱(MS)用于以下所述的实验中,以表征伴有和不伴有缀合的免疫刺激部分的流感-铁蛋白。在一些情况下,进行MS的分析物是流感-铁蛋白的胰蛋白酶消化物。胰蛋白酶通常会在赖氨酸和精氨酸残基之后切割,除非在之后是脯氨酸时不切割。然而,结构化的纳米颗粒(在所指示的胰蛋白酶位点的C末端)是抗蛋白水解的。在图5中指示了在天然条件下牛蛙-幽门螺杆菌铁蛋白构建体(如SEQ ID NO:14)中的可以被胰蛋白酶切割的最远(C末端)胰蛋白酶位点。因此,胰蛋白酶消化物释放出适于MS分析的抗蛋白水解的铁蛋白颗粒。

[0236] 因此,在接头序列是胰蛋白酶可接近的情况下,可以使用胰蛋白酶消化之后进行简化的MS分析(而不管未切割多肽中存在的N末端抗原如何),以评价与纳米颗粒的缀合。设计此方法以克服糖蛋白抗原对MS分析造成的复杂性。例如,H1/茎-Np原本只有在PNGase处理后才可以通过MS分析(图8A)。

[0237] 将来自以下流感株系的HA序列与幽门螺杆菌-牛蛙杂合铁蛋白的N末端在基因上融合,以构建HA-纳米颗粒:A/蒙茅斯堡/1-JY2/1947(GenBank CY147342,氨基酸1-518,Y108F);A/马来西亚/302/1954(GenBank CY009340.1,氨基酸1-518,Y108F);A/丹佛/1957(GenBank CY008988,氨基酸1-517,Y108F);A/香港/117/1977(GenBank CY009292,氨基酸1-518,Y108F);A/新喀里多尼亚/20/99(GenBank AHJ09883.1,氨基酸1-518);A/加利福尼亚/4/2009(GenBank AHJ09884.1,氨基酸1-518);COBRA P1(美国专利公开案US20150017196A1中的SEQ ID NO:2,氨基酸1-518,Y108F)和COBRA X6(美国专利公开案US20140127248A1,氨基酸1-517,Y108F)。COBRA P1和COBRA X6是通过分层序列平均化的计算方法产生的(Carter DM,等人,J Virol 90:4720-4734(2016))。COBRA X6是从跨越1999-2012年的人H1N1流感序列产生的,并且COBRA P1是从跨越1933-1957年和2009-2011年的人H1N1株系加上来自1931-1998年的猪H1N1流感株系产生的(Carter 2016)。将HA-铁蛋白基因克隆到SIB002载体的XbaI/BamHI位点中以进行哺乳动物表达,在ATG起始密码子前

面具有gccacc kozak序列。对所有序列进行密码子优化以在人细胞系中表达。

[0238] 将HA-Np质粒用Powerprep试剂盒(Origene目录号NP100009)纯化,并用于转染Expi293细胞(ThermoFisher目录号A14635)。将FectoPRO DNA转染试剂(Polyplus#116-100)在标准条件(0.5 μ g DNA/mL,0.75 μ l FectoPRO试剂/mL和0.45 μ l增强剂/mL)下使用。转染后4-6天,通过在4度下以3,488g离心15min从Expi293上清液收获纳米颗粒,并且将其通过0.45 μ m真空驱动过滤器单元(Thermo Scientific目录号167-0045)进行过滤。使HA-Np通过重力流通过Q-Sepharose快速流动柱(GE目录号17051001),并且收集流穿液并将其用水稀释3倍,并且通过添加终浓度为50mM的Tris缓冲液(pH 8.5)来调节pH;或将上清液用缓冲液A(50mM Tris pH 8.5,5mM NaCl)稀释5倍,而不使用初始Q-琼脂糖柱。然后将样品加载至Q-琼脂糖柱(HiTrap Q HP,GE目录号17115401),并在NaCl梯度上洗脱蛋白质,其中使用缓冲液A(50mM Tris pH 8.5,5mM NaCl)和缓冲液B(50mM Tris,pH 8.5,1M NaCl)的0%-60%混合,经30个柱体积。收集HA-纳米颗粒蛋白质级分,并用Amicon Ultra-15离心过滤器装置(Millipore目录号UFC910024)浓缩,并且通过尺寸排阻色谱用Superose 6柱PG XK 16/70,60-65cm(目录号:90100042)在磷酸盐缓冲液中进一步纯化。将最终的级分浓缩并通过0.22 μ m过滤器(Millipore SLGV004SL)过滤灭菌。使用无内毒素的溶液,并且使用带有LAL盒的Charles Rivers Endosafe PTS仪器对最终蛋白质进行测试,检测极限为0.05EU/ml。

[0239] 示出了H1/茎-Np(SEQ ID NO:43,图6A)和H5/COBRA-Np(SEQ ID NO:32,图6B)的胰蛋白酶消化。在两种情况下,在胰蛋白酶消化的情况下(以“+”表示)的分子量移位对应于SM7/8a的分子量(711道尔顿)。因此,这些数据表明SM7/8a与包含表面暴露的半胱氨酸的铁蛋白纳米颗粒成功缀合。

[0240] 在使用PNGase去除H1/茎-Np的糖基化而未经胰蛋白酶处理的凝胶移位实验(图7A)中,与低分子量分子(如马来酰亚胺-PEG4-SM7/8a)缀合后铁蛋白纳米颗粒重量变化的分辨率是不清楚的。如图7B所示,胰蛋白酶处理允许通过凝胶移位测定确认小分子如SM7/8a与H1/Stem-Np的缀合。可以通过去糖基化H1/Stem-Np的凝胶移位测定(图7A),以及还通过在天然条件下通过胰蛋白酶消化从H1/茎-Np释放的铁蛋白纳米颗粒的凝胶移位测定观察到更高分子量的分子(例如CpG)的缀合。因此,在天然条件下使用胰蛋白酶消化铁蛋白纳米颗粒提供一种表征与TLR激动剂缀合的核心片段的方法作为平台技术,所述核心片段原本可能难以在对于具有可能干扰凝胶移位或质谱(MS)实验的特性的给定抗原上通过这些分析测定来分析。

[0241] 将SM7/8a-PEG4-马来酰亚胺缀合至H1/茎-Np并仅出于分析目的进行PNGase处理之后,在MS上看到质量为42930Da的缀合产物,其比未缀合的H1/茎-Np重约715kDa(图8A)。胰蛋白酶消化后,减小的铁蛋白纳米颗粒的重量为19510Da(基于纳米颗粒的H1/茎部分的切割)(图8B)。缀合后,胰蛋白酶消化的缀合纳米颗粒的重量也更重约715kDa。因此,图8A和图8B二者均支持在将包含佐剂的接头与铁蛋白纳米颗粒缀合中约100%的缀合效率。

[0242] 在2步式点击化学反应中,将马来酰亚胺-PEG4-DBC0接头缀合至H1/茎-Np,其中马来酰亚胺与铁蛋白纳米颗粒上的表面暴露的半胱氨酸反应,在胰蛋白酶消化后通过MS分析看到与接头添加的分子量(大约675Da)一致的重量增加(图9)。

[0243] 接下来,将叠氮化物-CpG添加至H1/茎-Np-PEG4-DBC0中间体(在图10中标记为

“Ma1-PEG4-DBCO之后”), 将其通过凝胶移位测定来确认。叠氮化物-CpG从IDT订购(SEQ ID NO:45)。在SDS-PAGE之前, 将缀合物和对照用PNGase处理。与接头缀合相比, 由于增加的质量(约7.5kDa), CpG缀合引起向上的凝胶移位。在图10中示出了通过对标记为“茎-Np-CpG”和“茎-Np”的两个条带的密度测定而定量的50%缀合效率的例子。茎-Np是H1/茎-Np(SEQ ID NO:43)。未处理是指还原前的H1/茎-Np。TCEP还原的是指在室温下用3mM TCEP处理1小时并在4℃下用PBS透析过夜的H1/茎-Np。

[0244] 也将烟草蚀纹病毒(TEV)蛋白酶切割位点(SEQ ID NO:44)用于评价缀合。使用2步式点击化学方法, 将3M-012缀合至各种NC99HA-TEV-Np构建体(SEQ ID NO:9-12)。用2或10mM TCEP还原HA-TEV-Np构建体。通过透析(具有10kDa MWC0的3mL透析盒, Thermo#87730)至TRIS缓冲液(100mM TRIS pH 8.0, 50mM NaCl)中来去除TCEP。添加DBCO-PEG4-马来酰亚胺接头(Sigma#760676)。在也缓冲液交换至PBS 7.4中的同时, 用超滤(100kDa MWC0, Millipore Amicon#UFC810024或#UFC910096)去除过量的接头。最后, 将叠氮化物官能化的TLR激动剂(CpG或3M-012)点击到分子上。通过超滤(100kDa MWC0, Millipore Amicon#UFC810024或#UFC910096)或凝胶过滤去除过量的药物。HA-TEV-Np中的TEV切割位点允许TEV选择性切割以便分析缀合。

[0245] 在将3M-012(约500Da+约600Da接头)缀合至HA-TEV-Np构建体之后, 用TEV处理构建体。TEV切割后, 在凝胶移位SDS-PAGE上所得的HA-条带为约57kDa加上聚糖和Ferr约20kDa(图11, 标记为“+TEV”的泳道)。约20和21kDa的双条带(分别标记为“S26C”、“S72C”、“A75C”和“S111C”(分别为SEQ ID NO:10、11、12和9)的泳道)指示成功的缀合。缀合效力对应于条带强度, 其中较低的条带是未缀合的而上面的条带是缀合的铁蛋白。

[0246] 发现包含S111C的H1/茎-Np在293Expi细胞中表达时具有109道尔顿的翻译后修饰(与半胱氨酰化一致)(图12A), 所述翻译后修饰通过以下方式而去除: 在室温下用3mM浓度下的TCEP(三(2-羧乙基)膦)还原1小时以释放出反应性硫醇(图12B)。因此, 可以还原修饰以将表面暴露的半胱氨酸暴露为游离硫醇, 所述游离硫醇对商业接头或定制的小分子上的马来酰亚胺基团具有高反应性。

[0247] MS数据也确认了3M-012与HA-Np的成功缀合。HA-TEV-Np-S26C(SEQ ID NO:10;图12C)、A75C(SEQ ID NO:12;图12D)或S111C(SEQ ID NO:9;图12E)。包含A75C和S111C铁蛋白修饰的纳米颗粒(分别为SEQ ID NO:12和9)显示出与3M-012的大于95%缀合率。因此, MS数据指示, 佐剂可以与由铁蛋白中突变产生的各种表面暴露的半胱氨酸连接, 从而成功产生与佐剂连接的抗原性铁蛋白多肽。

[0248] 负染色电子显微镜(EM)确认缀合不会影响纳米颗粒完整性。示出了单独的H1/茎-Np(SEQ ID NO:43)(图13A)、与马来酰亚胺-PEG4-SM7/8a缀合的H1/茎-Np(图13B)、与CpG缀合的H1/茎-Np(图13C)、单独的NC99 HA-Np(SEQ ID NO:9)(图13D), 经由DBCO-马来酰亚胺接头与3M-012缀合的NC99 HA-Np(图13E)和经由DBCO-马来酰亚胺接头与CpG缀合的NC99 HA-Np(图13F)。此外, 与未缀合的H1/茎-Np相比(图14C), 将TLR7/8激动剂(马来酰亚胺-PEG4-SM7/8a, 图14A)或经由马来酰亚胺-PEG4-DBCO接头将TLR9激动剂(CpG, 图14B)缀合至H1/茎-Np不影响纳米颗粒完整性, 如通过动态光散射(DLS)测量的。

[0249] 这些数据证明了包含铁蛋白和HA多肽的抗原性多肽的成功制备。此外, 可以将包含佐剂的接头成功地缀合至这些铁蛋白纳米颗粒。

2. 对HA-铁蛋白纳米颗粒组合物的免疫原性的表征

[0250] 评估了各种佐剂和将TLR激动剂与H1/茎-Np缀合对H1/茎-Np组合物的免疫原性的影响。图15A示出了通过用H1/茎-Np TLR7/8-激动剂缀合物免疫诱导的针对H1/新喀里多尼亚/1999 (NC99) HA三聚体的IgG抗体效价。在第0周和第3周用10 μ g H1/茎-Np进行给药,将所述H1/茎-Np在不伴有佐剂的情况下、在伴有等摩尔掺合的游离SM7/8a佐剂(83.3ng)的情况下、在伴有掺合的高剂量游离SM7/8a佐剂(21.84 μ g)的情况下、或在伴有1:1体积比的PAA或AF03佐剂的情况下进行给予。在不存在任何其他佐剂的情况下给予H1/茎-Np-SM7/8a缀合物。在第二次剂量后2周测定血清。图15B出了通过用H1/茎-Np TLR9-激动剂缀合物免疫诱导的针对H1/茎三聚体的IgG抗体效价。在第0周和第3周用10 μ g H1/茎-Np进行给药,将所述H1/茎-Np在不伴有佐剂的情况下、在伴有等摩尔掺合的游离CpG佐剂(850ng)的情况下、在伴有掺合的高剂量游离CpG佐剂(20 μ g)的情况下、或在伴有1:1体积比的AF03佐剂的情况下进行给予。在不存在任何其他佐剂的情况下给予H1/茎-Np-CpG缀合物。在第二次剂量后2周测定血清。

[0251] 图15A中的数据表明,SM7/8a-缀合的H1/茎-Np显示出与掺合有聚丙烯酸(PAA)或AF03佐剂的未缀合的纳米颗粒可比的免疫原性。直接缀合至铁蛋白纳米颗粒的SM7/8a比作为与铁蛋白纳米颗粒分开的化合物给予的等摩尔剂量的SM7/8a(“等摩尔混合”)更有效。此外,直接缀合至铁蛋白纳米颗粒的SM7/8比作为与铁蛋白纳米颗粒分开的化合物给予的更高剂量的SM7/8a(“高混合”)更有效。因此,SM7/8a与包含HA多肽和铁蛋白的抗原性多肽的缀合使得所述化合物具有免疫原性和自我佐剂化。给予自佐剂化组合物可以允许减少或消除单独的佐剂。类似地,图15B表明,与未缀合的和等摩尔掺合的对照相比,TLR9激动剂CpG与H1/茎-Np的缀合将其免疫原性改善至与给予23倍高剂量的掺合CpG分子可比的水平。

[0252] 还在体外评估了血清中和H1/新喀里多尼亚/1999 (NC99) HA/NA假型化慢病毒的能力。如先前所述进行假型化中和测定(Kanekiyo等人,Nature 499:102-106(2013))。简而言之,通过用Profection哺乳动物转染试剂盒(Promega目录号E1200)转染5种质粒,即400ng HA、100ng NA、50ng TMPRSS2、7 μ g CMV Δ R8.2和7 μ g pHR'CMV-Luc,将慢病毒包装在HEK293T细胞中。转染后48和72小时收集病毒颗粒,并且将其通过0.45 μ m过滤器过滤。

[0253] 以三周的间隔对小鼠(n=5/组)免疫两次。将与3M-012缀合的NC99 HA-Np (SEQ ID NO:9) (HA-Ferr-3M-012,0.22 μ g/剂量)与以下掺合对照平行给予:NC99 HA-Np(0.22 μ g/剂量)和10 μ g 3M-012(典型的治疗剂量)的混合物,以及单独地,NC99 HA-Np(0.22 μ g/剂量)和1.7ng 3M-012(缀合物的等摩尔匹配物)的混合物。另外的对照是以匹配的HA含量(0.17 μ g HA/剂量)投配的未缀合NC99 HA-Np和灭活流感疫苗(IIV)。在第5周,测定来自这些小鼠的血清的连续稀释物对来自所指示株系的HA和神经氨酸酶(NA)基因假型化的慢病毒的中和活性。将一系列稀释度下的抗血清与固定量的慢病毒一起预孵育,并用于感染靶293A细胞。72小时后用Promega萤光素酶测定系统(目录号E1500)定量感染。使用Graphpad Prism软件根据这些中和曲线计算IC₅₀值,以确定达到50%PsV中和的血清稀释因子。还分别在第5周和第8周测定终点效价(ELISA)和血凝抑制(HAI)效价。关于示例性ELISA和HAI程序,参见以下实施例。一式三份地运行所有样品。

[0254] 基于ELISA终点效价(图16A)和假病毒中和IC₅₀效价(图16B),与匹配的掺合物、未缀合的颗粒或IIV相比,与3M-012缀合的NC99 HA-Np诱导更强的结合和中和抗体应答。HA-

Np-3M-012缀合物还诱导了比等摩尔掺合物对照显著更强的HAI效价(图16C)。另外,HAI效价比未缀合的HA-Ferr和IIV高3.7和1.6倍,但是这些结果并不显著。

[0255] 与掺合对照(HA-Np(0.22 μ g/剂量)和20 μ g CpG(典型的治疗剂量)的混合物,以及HA-ferr(0.22 μ g/剂量)和21ng CpG(缀合物的等摩尔匹配物)的混合物)相比,还评估了与CpG缀合的NC99 HA-Np(SEQ ID NO:9)(HA-Ferr-CpG,0.22 μ g/剂量)的免疫原性。另外的对照是以匹配HA含量(0.17 μ g HA/剂量)投配的未缀合HA-Np和IIV。如上所述,在第0周和第3周给药。ELISA和PsV测定是对于加强免疫后2周的血清进行的,并且在加强免疫后5周对血清进行HAI。一式三份地运行所有样品。

[0256] 基于ELISA终点效价(图17A)或假病毒中和IC50效价(图17B),与匹配的掺合物、未缀合的颗粒或IIV相比,缀合物诱导更强的结合和中和抗体应答。HA-Np-CpG缀合物诱导了比等摩尔掺合物对照显著更强的HAI效价(图17C)。另外,HAI效价比未缀合的HA-Ferr和IIV高2.6和3.0倍,但是这些结果并不显著。

[0257] 还在非人灵长类动物模型中评估了H1/茎-Np-TLR激动剂缀合物的免疫原性。将十二只食蟹猴(长尾猕猴)由Bioqual公司依照所有联邦法规(包括USDA法规和动物福利法)饲养和照管。对动物进行预筛选并且通过ELISA选择对2016-2017年Fluzone四价抗原(A/加利福尼亚/07/2009;A/香港/4801/2014X-263B;B/普吉岛/3073/2013;B/布里斯班/60/2008)缺乏反应性的动物。将5至13岁、体重3.5kg至7.8kg的NHP受试者(10只雌性,2只雄性)随机分配到每个免疫组(n=3只动物/组)。使每个组在第0、4和10周接受三次剂量:50 μ g伴有AF03的H1-SS-np或200 μ g H1-SS-np(无佐剂对照)、或200 μ g H1-SS-np-SM7/8缀合物、或200 μ g H1-SS-np-CpG。在第0、2、4、6、8、10和12周收集10mL血液样品用于血清分离。在第0、2、5、6和12周收集10-16mL的血液样品用于PBMC分离。按照标准操作程序分离并低温保存血清和PBMC。食蟹猴被分配为在第0、4和10周用以下项免疫:50 μ g与掺合的AF03佐剂一起配制的H1/茎-Np、或200 μ g H1/茎-Np(无佐剂对照)、或200 μ g H1/茎-Np-SM7/8a缀合物(图3A中所示的)或200 μ g H1/茎-Np-CpG缀合物(图3B中所示的)。测定在各个时间点分离的血清的根据ELISA得出的抗HA抗体效价(图25A),以及所述血清对用H1亚型HA和NA(图25B)或用H5亚型HA和NA亚型(图25C)假型化的慢病毒的中和作用。合在一起,图25A至图25C中所示的结果证明,TLR激动剂的缀合改善了H1/茎-Np在非人灵长类动物模型中的免疫原性。在此模型中,TLR7/8激动剂缀合物显现比CpG缀合物产生更高的效价,并且AF03掺合佐剂在测试条件下引发最高的效价。

[0258] 接下来,确定当以较高剂量给予时3M012缀合物的免疫原性是否可以改善。为此,以0.1 μ g、0.5 μ g、2.5 μ g和12.5 μ g剂量的HA-NP测试缀合物、等摩尔掺合物、较高剂量掺合物和未缀合的纳米颗粒(图26A至图26B)。在0.1 μ g和0.5 μ g HA-NP剂量下,在中和和HAI测定中看到最小应答,除了高掺合物剂量。在2.5 μ g剂量下,未缀合的HA-NP以及HA-NP与3M012的等摩尔掺合物二者诱导了最小的抗体应答。与高剂量掺合物组相比,3M012缀合的HA-NP尽管含有少>5000倍的3M012,但诱导相似的抗体应答,这证明了直接缀合和TLR激动剂靶向递送的功效。

3. 通过EM和DLS对包含候选多肽的纳米颗粒的表征

[0259] 使用上文概述的方法表达图1A中所呈现的包含流感多肽的HA-Np,并将其释放到培养基中(在图1A中示出HA部分的序列比对和在图1B中示出系统树图)。图1B中的箭头指示

候选多肽。通过阴离子交换和尺寸排阻色谱从293Expi细胞培养上清液中纯化图18A中列出的HA-Np。在图18B中示出了确认纳米颗粒的成功产物的考马斯染色结果。

[0260] 电子显微镜(EM)和动态光散射(DLS)分析如下进行。

[0261] 动态光散射是在25℃下在Wyatt的DynaPro板读取器II上测量的。纯化的HA-Np显示出根据动态光散射在16与18纳米之间的预期大小(图18A,通过“大小”度量指示)。还通过负染色透射电子显微镜(TEM)确认了纳米颗粒的形成(图18C)。对于EM,将HA-铁蛋白纳米颗粒样品吸附至碳涂覆网格持续1分钟,所述碳涂覆网格已通过暴露于辉光放电30秒而变得亲水。用滤纸(Whatman#1)去除过量的液体,并用0.75%甲酸铀酰将样品染色30秒。用滤纸去除多余的甲酸铀酰后,在TecnaiG² Spirit BioTWIN中检查网格,并使用AMT 2k CCD相机记录图像。

4. 单一株系HA-铁蛋白纳米颗粒针对一组代表性的相异H1N1流感病毒的免疫原性

[0262] 为了研究来自特定病毒株系的HA-Np的免疫原性,用来自目的株系的纳米颗粒对小鼠免疫两次,并在第二次免疫后3周使用血凝素抑制测定(HAI)来测定血清。所有免疫均遵循用于动物处理的既定准则。在第0周和第3周,用220ng HA-铁蛋白纳米颗粒(HA含量为170ng HA)免疫Balb/C小鼠(5只/组),并且所述HA-铁蛋白纳米颗粒在适用时在临肌内注射(每只后肢50μL)之前与佐剂1:1混合。如图例中所指示,使用Ribi(Sigma Adjuvant System,目录号S6322-1vl)或AF03(Sanofi Pasteur)。对于二价、三价和四价组合,在注射前将220ng的每种纳米颗粒预先混合。在加强免疫注射后2周和3周收集血清。

[0263] HA抑制测定(HAI)使用的流感种子原液来自美国疾病控制与预防中心(Centers for Disease Control and Prevention)(美国乔治亚州亚特兰大)。通过用三份酶稀释一份血清用受体破坏酶(RDE)对免疫血清进行预处理,并且在37℃水浴中孵育过夜。通过在56℃下孵育30分钟、之后添加六份PBS至1/10的最终稀释度来使酶失活。在V型底96孔微量滴定板中进行HAI测定,所述微量滴定板具有在0.5%火鸡红细胞中的4个病毒HA单位(HAU)。将HAI效价确定为导致血凝完全抑制的最高血清稀释度。

[0264] 对于所有数据,误差棒表示通过测定来自给定治疗组中每只动物的样品获得的平均值的标准误差;对于小鼠,n=5,并且对于雪貂,n=12。学生T检验是使用Microsoft Excel计算的。方差分析是使用VassarStats(可通过网站在vassarstats.net/anova1u.html获得)计算的。

[0265] 使用了跨越78年的病毒进化的一组16种代表性流感株系。所述组中的病毒列于表2中。

表2:用于HAI的H1N1流感株系组

H1N1 HAI流感组
A/波多黎各/1934
A/Weiss/1/1943
A/FM/1/1947
A/丹佛/1/1957
A/新泽西州/8/1976
A/USSR/90/1977
A/巴西/11/1978

A/智利/1/1983
A/台湾/1/1986
A/德克萨斯州/36/1991
A/北京/262/1995
A/新喀里多尼亚/20/1999
A/所罗门群岛/6/2006
A/布里斯班/59/2007
A/加利福尼亚/07/2009
A/孟加拉国/2021/2012
A/越南/3050/2013

[0266] 在所有情况 (CA09、NC99、FM47和HK77, 图19A至图19D) 下均观察到匹配株系的强效中和。

[0267] 也通过ELISA确认了针对匹配抗原的血清学应答 (图20A至图20F)。给予至每个组的免疫原列于图20A中。将三聚体用于ELISA, 如通过图20B至图20F所指示的。

[0268] 对于ELISA测定, 将Nunc MaxiSorp 96孔板 (目录号44-2404-21) 在4°C下用100ng/孔三聚体HA或稳定化的茎蛋白包被过夜, 并用在PBST中的5%脱脂乳封闭。如所指示那样稀释抗血清并在室温下孵育1小时, 并且用也在室温下孵育1小时的在5%乳-PBST中的抗小鼠HRP抗体 (目录号NA931, 在1:5,000下) 或抗猴HRP抗体 (Southern Biotech目录号4700-05, 批号A3814-P907, 在1:5,000下) 检测结合的抗体。用PBST洗涤5次后, 用SureBlueTMB底物 (目录号52-00-02) 对HRP进行显色, 并用0.5N硫酸终止。在450nm (Spectramax M5) 下读取吸光度。终点效价是使用Graphpad prism以0.2的阈值和0.05的典型背景水平计算的。

[0269] 还如上所述那样评价HA/NA假型化慢病毒的中和作用, 并将结果示于表3中。在所有测试的情况下, 对于匹配株系均观察到强的中和活性, 并将这些值用作阈值。具有互补中和活性的HA-Np的组合以加和方式导致扩大的交叉反应性。

表3: 用作为单一组分或组合给予的HA-铁蛋白纳米颗粒进行两次免疫后, 小鼠中针对HA/NA假型化慢病毒 (PsV) 的中和IC50

组 编 号	免疫原	FM	马来西亚	香港	新喀里多 尼亚
		1947	1954	1977	1999
1	NC99 Np	<200	<200	1.8×10^3	5.8×10^4
2	CA09 Np	<200	<200	8.8×10^2	5.3×10^2
3	FM47 Np	4.2×10^3	2.1×10^4	2.8×10^4	4.8×10^2
4	HK77 Np	1.2×10^5	1.7×10^4	4.6×10^3	9.9×10^2
5	Mal54 Np	4.3×10^3	5.7×10^3	6.8×10^3	2.8×10^3
6	NC99+CA09 Np	<200	<200	2.1×10^3	6.2×10^4
7	NC99+CA09+FM47 Np	4.4×10^5	1.6×10^3	2.3×10^4	2.9×10^3
8	NC99+CA09+HK77 Np	2.8×10^5	8.9×10^3	2.1×10^5	1.5×10^3
9	NC99+CA09+Mal54 Np	1.7×10^3	1.6×10^5	2.2×10^3	1.7×10^3
10	NC99+CA09+HK77+FM47 Np	4.1×10^5	2.6×10^3	2.1×10^5	1.4×10^3
11	NC99+CA09+HK77+MAL54 Np	7.4×10^4	1.5×10^5	1.6×10^5	2.7×10^3
12	NC99+CA09+FM47+MAL54 Np	3.7×10^5	1.1×10^5	3.1×10^4	9.8×10^4
13	NC99 IIV	1.0×10^3	<200	1.6×10^3	4.8×10^4
14	CA09 IIV	<200	<200	1.6×10^3	1.0×10^3
15	NC99+CA09 IIV	1.0×10^3	<200	1.7×10^3	3.0×10^4

<10,000 >10,000 >100,000

正常字体 粗字体 斜体字体

[0270] CA09 HA-Np引发了强的免疫应答,但这些限于当代(2009年后)株系和也源自猪并且与CA09具有紧密同源性的1976年分离物(图19A)(参见Gaydos等人2006. *Emerg Infect Dis* 12:23-28(1976))。NC99 HA-Np引发了针对从1990年代末至2000年代初的流感病毒的强效中和作用(图19B)。对FM47 HA-Np的免疫应答主要扩展到匹配的株系,但是它还显示出与1977年株系的中度交叉反应性(图19C)。这种水平的交叉反应性与1977年暴发(其主要影响26岁以下的人群)具有临床相关性,这表明暴露于来自1940-50年暴发的流感病毒赋予了针对1977年株系的保护(Kilbourne 2006)。有趣的是,在HA序列中, HK77 HA-Np脱颖而出,因为其交叉反应性扩展到来自1947、1978和1983年的株系(图19D)。另一方面,对MAL54 HA-Np和DV57 HA-Np的免疫应答限于匹配的株系(图19E、图19F和表3)。

[0271] 用COBRA P1和COBRA X6纳米颗粒观察到的交叉反应性与其病毒样颗粒(VLP)对应物一致(参见Carter DM等人, *J Virol* 90:4720-4734(2016))。由COBRA P1 HA-Np引发的免疫应答与CA09 HA-Np相似(图19A和图19G),并且COBRA X6 HA-Np显示出与NC99 HA-Np相似的免疫概况(图19B和图19H)。

5. HA-铁蛋白纳米颗粒的单价、二价、三价和四价配制品

[0272] 评价了由选择的HA-Np的组合引发的HAI交叉反应性。用通过组合单独的纳米颗粒制备的二价、三价或四价配制品如上所述那样免疫和测试小鼠。相对于任一种单价组合物, NC99和CA09 HA-Np的二价组合显示出扩大的交叉反应性(图21A)。然而,此二价组合并未引发针对来自1934-1957年和1977-1991年的较早相异株系的可检测抗体效价。COBRA X6和COBRA P1二价组合的免疫原性遵循相同趋势(图21B)。与NC99/CA09二价组合物相比,此组合显示出增加的广度,但针对若干种株系的HAI效价是中等的。对于三价组合,当第三组分是FM47 HA-Np(图21C)或HK77 HA-Np(图21E)时,将第三组分包含到NC99和CA09 HA-Np中会增加交叉反应性,但MAL54HA-Np(图21D)并未增强广度。与NC99、CA09和HK77 HA-Np的三价

组合相比,在四价配制品中添加第四组分不会导致另外的可观察到的交叉反应性(图21F至图21H)。

[0273] 当使用不同的佐剂(即,Ribi对比AF03,图22A至图22F)时观察到可比较结果。重要的是,没有证据表明由共同给予不同的HA-Np引起的抗原性竞争。这些数据表明,对于在其单独的HAI概况中存在高度互补性的情况,交叉反应性概况是加和性的。还测量了用作为使用归一化剂量的HA的由蛋产生的灭活流感疫苗(IIV)递送的NC99和CA09免疫原获得的HAI概况(图23A至23C)。

6. 在雪貂中用纳米颗粒组合物进行的针对攻击的保护

[0274] 在与人类疾病相关的动物模型雪貂中测试了某些HA-Np组合的功效。用磷酸盐缓冲液(图24A)、CA09灭活的流感疫苗(IIV,图24B)、野生型HA-Np的三价组合(NC99+CA09+HK77,图24C)或COBRA P1+C0 BRA X6+HK77 HA-Np的组合(图24D)对雪貂(n=12/组)进行免疫。肌肉注射之前,将这些组合物与AF03佐剂1:1混合,实现1ml最终注射体积。

[0275] 两次免疫后,观察到针对匹配的株系的显著HAI效价(图24B至图24D)。用纳米颗粒组合免疫的两组雪貂均显示出针对FM47、HK77和NC99的显著HAI效价。如在小鼠中观察到的,CA09 IIV未在雪貂中引发针对FM47和HK77株系的交叉中和效价(图24B)。

[0276] 免疫后,将雪貂用不匹配的相异株系H1/蒙茅斯堡/1947病毒攻击。在第二次免疫后4周通过以下方式进行流感攻击:用1ml的A/蒙茅斯堡/1/1947病毒以50%组织培养物感染剂量(TCID₅₀)的 $10^{4.65}$ 倍进行鼻腔接种。每日跟踪临床症状,持续2周,并且在攻击后7天内每日收集鼻洗液并且通过标准TCID₅₀测定测试其病毒载量。攻击后,从鼻洗液中定量病毒效价。接受三价NC99+CA09+HK77或COBRA-P1+X6+HK77纳米颗粒组合的雪貂群组比对照组更快地清除病毒,从而在感染后第5天显示出显著降低的病毒效价(图24E, $p \leq 0.001$)。相比之下,CA09 IIV免疫组未比PBS免疫对照组显著更快地清除病毒。在所有组中,FM47株系成功复制,并在感染后不超出一周被清除。因此,在与人类疾病相关的感染动物模型中,三价组合刺激了也针对相异病毒攻击进行保护的有效HAI应答。

序列表

<110> 赛诺菲

<120> 抗原性流感-铁蛋白多肽

<130> 01121-0034-00PCT

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<150> 62/652,204

<151> 2018-04-03

<160> 231

<170> PatentIn 3.5版

<210> 1

<211> 685

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 1

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Met Lys Ala Lys Leu Leu Val Leu Leu Cys Thr Phe Thr Ala Thr Tyr
1           5           10           15
Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
          20           25           30
Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
          35           40           45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile
          50           55           60
Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly
65           70           75           80
Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile
          85           90           95
Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe
          100          105          110
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
          115          120          125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr
          130          135          140
Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe

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145	150	155	160
Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn			
	165	170	175
Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val Leu			
	180	185	190
Trp Gly Val His His Pro Pro Asn Ile Gly Asn Gln Arg Ala Leu Tyr			
	195	200	205
His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg			
	210	215	220
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu			
225	230	235	240
Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile			
	245	250	255
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe Ala			
	260	265	270
Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Pro Met			
	275	280	285
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser			
	290	295	300
Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro			
305	310	315	320
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn			
	325	330	335
Ile Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe			
	340	345	350
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His			
	355	360	365
His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr			
	370	375	380
Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu			
385	390	395	400
Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu			
	405	410	415
Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu			
	420	425	430
Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu			
	435	440	445
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys			
	450	455	460

Val	Lys	Ser	Gln	Leu	Lys	Asn	Asn	Ala	Lys	Glu	Ile	Gly	Asn	Gly	Cys
465					470					475					480
Phe	Glu	Phe	Tyr	His	Lys	Cys	Asn	Asn	Glu	Cys	Met	Glu	Ser	Val	Lys
			485						490					495	
Asn	Gly	Thr	Tyr	Asp	Tyr	Pro	Lys	Tyr	Ser	Glu	Glu	Ser	Lys	Leu	Asn
		500						505					510		
Arg	Glu	Lys	Ile	Asp	Ser	Gly	Gly	Asp	Ile	Ile	Lys	Leu	Leu	Asn	Glu
	515						520					525			
Gln	Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met	Ser
	530					535					540				
Ser	Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu	Phe
545					550				555						560
Asp	His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe
			565						570					575	
Leu	Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	Ala	Pro
		580						585					590		
Glu	His	Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr	Glu
	595						600					605			
His	Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	His	Ala
	610					615					620				
Ile	Lys	Cys	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr	Val
625					630				635						640
Ala	Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp	Lys
			645						650					655	
Ile	Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	Asp	Gln
		660						665					670		
Tyr	Val	Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser	Gly	Ser			
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20	25	30
Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn		
35	40	45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile		
50	55	60
Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly		
65	70	75
Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile		
85	90	95
Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe		
100	105	110
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe		
115	120	125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr		
130	135	140
Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe		
145	150	155
Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn		
165	170	175
Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val Leu		
180	185	190
Trp Gly Val His His Pro Pro Asn Ile Gly Asn Gln Arg Ala Leu Tyr		
195	200	205
His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg		
210	215	220
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu		
225	230	235
Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile		
245	250	255
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe Ala		
260	265	270
Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Pro Met		
275	280	285
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser		
290	295	300
Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro		
305	310	315
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn		

	325		330		335
Ile Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe					
	340		345		350
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His					
	355		360		365
His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr					
	370		375		380
Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu					
385		390		395	400
Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu					
	405		410		415
Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu					
	420		425		430
Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu					
	435		440		445
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys					
	450		455		460
Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys					
465		470		475	480
Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys					
	485		490		495
Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn					
	500		505		510
Arg Glu Lys Ile Asp Gly Ser Glu Ser Gln Val Arg Gln Gln Phe Ser					
	515		520		525
Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln					
	530		535		540
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser					
545		550		555	560
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr					
	565		570		575
Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro					
	580		585		590
Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu					
	595		600		605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu					
	610		615		620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp His Ala					
625		630		635	640

Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu
 645 650 655
 Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu
 660 665 670
 Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys
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 35 40 45
 Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile
 50 55 60
 Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly
 65 70 75 80
 Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile
 85 90 95
 Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe
 100 105 110
 Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
 115 120 125
 Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr
 130 135 140
 Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe
 145 150 155 160
 Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn
 165 170 175

Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val Leu		
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Trp Gly Val His His Pro Pro Asn Ile Gly Asn Gln Arg Ala Leu Tyr		
195	200	205
His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg		
210	215	220
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu		
225	230	235
Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile		
245	250	255
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe Ala		
260	265	270
Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Pro Met		
275	280	285
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser		
290	295	300
Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro		
305	310	315
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn		
325	330	335
Ile Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe		
340	345	350
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His		
355	360	365
His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr		
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Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu		
385	390	395
Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu		
405	410	415
Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu		
420	425	430
Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu		
435	440	445
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys		
450	455	460
Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys		
465	470	475
Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys		

	485		490		495
Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn					
	500		505		510
Arg Glu Lys Ile Asp Gly Ser Glu Ser Gln Val Arg Gln Gln Phe Ser					
	515		520		525
Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln					
	530		535		540
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser					
545		550		555	560
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr					
	565		570		575
Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro					
	580		585		590
Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu					
	595		600		605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu					
	610		615		620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Ser Lys Asp His Ala					
625		630		635	640
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu					
	645		650		655
Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu					
	660		665		670
Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys					
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Val Asp Thr Val Leu Glu Lys Asn	Val Thr Val Thr His Ser Val Asn	
35	40	45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile		
50	55	60
Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly		
65	70	75
Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile		
85	90	95
Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe		
100	105	110
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe		
115	120	125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr		
130	135	140
Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe		
145	150	155
Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn		
165	170	175
Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val Leu		
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Trp Gly Val His His Pro Pro Asn Ile Gly Asn Gln Arg Ala Leu Tyr		
195	200	205
His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg		
210	215	220
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu		
225	230	235
Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile		
245	250	255
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe Ala		
260	265	270
Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Pro Met		
275	280	285
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser		
290	295	300
Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro		
305	310	315
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn		
325	330	335

Ile Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe		
340	345	350
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His		
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His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr		
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Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu		
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Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu		
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Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu		
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Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu		
435	440	445
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys		
450	455	460
Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys		
465	470	475
Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys		
485	490	495
Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn		
500	505	510
Arg Glu Lys Ile Asp Gly Ser Glu Ser Gln Val Arg Gln Gln Phe Ser		
515	520	525
Lys Asp Ile Glu Lys Leu Leu Asn Cys Gln Val Asn Lys Glu Met Gln		
530	535	540
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser		
545	550	555
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr		
565	570	575
Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro		
580	585	590
Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu		
595	600	605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu		
610	615	620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Ser Lys Asp His Ala		
625	630	635
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu		

645	650	655
Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu		
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Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys		
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Ser Arg Lys Ser		
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Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn		
35 40 45		
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile		
50 55 60		
Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly		
65 70 75 80		
Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile		
85 90 95		
Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe		
100 105 110		
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe		
115 120 125		
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr		
130 135 140		
Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe		
145 150 155 160		
Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn		
165 170 175		
Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val Leu		

			180				185				190				
Trp	Gly	Val	His	His	Pro	Pro	Asn	Ile	Gly	Asn	Gln	Arg	Ala	Leu	Tyr
			195				200				205				
His	Thr	Glu	Asn	Ala	Tyr	Val	Ser	Val	Val	Ser	Ser	His	Tyr	Ser	Arg
			210				215				220				
Arg	Phe	Thr	Pro	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	Arg	Asp	Gln	Glu
225			230				235				240				
Gly	Arg	Ile	Asn	Tyr	Tyr	Trp	Thr	Leu	Leu	Glu	Pro	Gly	Asp	Thr	Ile
			245				250				255				
Ile	Phe	Glu	Ala	Asn	Gly	Asn	Leu	Ile	Ala	Pro	Trp	Tyr	Ala	Phe	Ala
			260				265				270				
Leu	Ser	Arg	Gly	Phe	Gly	Ser	Gly	Ile	Ile	Thr	Ser	Asn	Ala	Pro	Met
			275				280				285				
Asp	Glu	Cys	Asp	Ala	Lys	Cys	Gln	Thr	Pro	Gln	Gly	Ala	Ile	Asn	Ser
290			295				300								
Ser	Leu	Pro	Phe	Gln	Asn	Val	His	Pro	Val	Thr	Ile	Gly	Glu	Cys	Pro
305			310				315				320				
Lys	Tyr	Val	Arg	Ser	Ala	Lys	Leu	Arg	Met	Val	Thr	Gly	Leu	Arg	Asn
			325				330				335				
Ile	Pro	Gln	Arg	Glu	Thr	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe
			340				345				350				
Ile	Glu	Gly	Gly	Trp	Thr	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	Tyr	His
			355				360				365				
His	Gln	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Gln	Lys	Ser	Thr
370			375				380								
Gln	Asn	Ala	Ile	Asn	Gly	Ile	Thr	Asn	Lys	Val	Asn	Ser	Val	Ile	Glu
385			390				395				400				
Lys	Met	Asn	Thr	Gln	Phe	Thr	Ala	Val	Gly	Lys	Glu	Phe	Asn	Lys	Leu
			405				410				415				
Glu	Arg	Arg	Met	Glu	Asn	Leu	Asn	Lys	Lys	Val	Asp	Asp	Gly	Phe	Leu
			420				425				430				
Asp	Ile	Trp	Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Glu
			435				440				445				
Arg	Thr	Leu	Asp	Phe	His	Asp	Ser	Asn	Val	Lys	Asn	Leu	Tyr	Glu	Lys
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Val	Lys	Ser	Gln	Leu	Lys	Asn	Asn	Ala	Lys	Glu	Ile	Gly	Asn	Gly	Cys
465			470				475				480				
Phe	Glu	Phe	Tyr	His	Lys	Cys	Asn	Asn	Glu	Cys	Met	Glu	Ser	Val	Lys
			485				490				495				

Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn			
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Arg Glu Lys Ile Asp Gly Ser Glu Ser Gln Val Arg Gln Gln Phe Ser			
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Ser Ser Asn Leu Tyr Met Cys Met Ser Ser Trp Ser Tyr Thr His Ser			
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Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro			
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Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu			
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Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu			
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Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Ser Lys Asp His Ala			
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Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu			
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Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu			
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35				40				45							
Leu	Leu	Glu	Asp	Ser	His	Asn	Gly	Lys	Leu	Cys	Leu	Leu	Lys	Gly	Ile
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Ala	Pro	Leu	Gln	Leu	Gly	Asn	Cys	Ser	Val	Ala	Gly	Trp	Ile	Leu	Gly
65				70				75				80			
Asn	Pro	Glu	Cys	Glu	Leu	Leu	Ile	Ser	Lys	Glu	Ser	Trp	Ser	Tyr	Ile
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Val	Glu	Thr	Pro	Asn	Pro	Glu	Asn	Gly	Thr	Cys	Phe	Pro	Gly	Tyr	Phe
100				105				110							
Ala	Asp	Tyr	Glu	Glu	Leu	Arg	Glu	Gln	Leu	Ser	Ser	Val	Ser	Ser	Phe
115				120				125							
Glu	Arg	Phe	Glu	Ile	Phe	Pro	Lys	Glu	Ser	Ser	Trp	Pro	Asn	His	Thr
130				135				140							
Val	Thr	Gly	Val	Ser	Ala	Ser	Cys	Ser	His	Asn	Gly	Lys	Ser	Ser	Phe
145				150				155				160			
Tyr	Arg	Asn	Leu	Leu	Trp	Leu	Thr	Gly	Lys	Asn	Gly	Leu	Tyr	Pro	Asn
165				170				175							
Leu	Ser	Lys	Ser	Tyr	Val	Asn	Asn	Lys	Glu	Lys	Glu	Val	Leu	Val	Leu
180				185				190							
Trp	Gly	Val	His	His	Pro	Pro	Asn	Ile	Gly	Asn	Gln	Arg	Ala	Leu	Tyr
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His	Thr	Glu	Asn	Ala	Tyr	Val	Ser	Val	Val	Ser	Ser	His	Tyr	Ser	Arg
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Arg	Phe	Thr	Pro	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	Arg	Asp	Gln	Glu
225				230				235				240			
Gly	Arg	Ile	Asn	Tyr	Tyr	Trp	Thr	Leu	Leu	Glu	Pro	Gly	Asp	Thr	Ile
245				250				255							
Ile	Phe	Glu	Ala	Asn	Gly	Asn	Leu	Ile	Ala	Pro	Trp	Tyr	Ala	Phe	Ala
260				265				270							
Leu	Ser	Arg	Gly	Phe	Gly	Ser	Gly	Ile	Ile	Thr	Ser	Asn	Ala	Pro	Met
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Asp	Glu	Cys	Asp	Ala	Lys	Cys	Gln	Thr	Pro	Gln	Gly	Ala	Ile	Asn	Ser
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Ser	Leu	Pro	Phe	Gln	Asn	Val	His	Pro	Val	Thr	Ile	Gly	Glu	Cys	Pro
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Lys	Tyr	Val	Arg	Ser	Ala	Lys	Leu	Arg	Met	Val	Thr	Gly	Leu	Arg	Asn
325				330				335							
Ile	Pro	Gln	Arg	Glu	Thr	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe

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Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His		
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His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr		
370	375	380
Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu		
385	390	395
Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu		
405	410	415
Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu		
420	425	430
Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu		
435	440	445
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys		
450	455	460
Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys		
465	470	475
Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys		
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Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn		
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Arg Glu Lys Ile Asp Gly Ser Glu Ser Gln Val Arg Gln Gln Phe Ser		
515	520	525
Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln		
530	535	540
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser		
545	550	555
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr		
565	570	575
Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro		
580	585	590
Val Gln Leu Thr Cys Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu		
595	600	605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu		
610	615	620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Ser Lys Asp His Ala		
625	630	635
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu		
645	650	655

Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu
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 35 40 45
 Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile
 50 55 60
 Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly
 65 70 75 80
 Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile
 85 90 95
 Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe
 100 105 110
 Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
 115 120 125
 Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr
 130 135 140
 Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe
 145 150 155 160
 Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn
 165 170 175
 Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val Leu
 180 185 190

Trp Gly Val His His Pro Pro Asn Ile Gly Asn Gln Arg Ala Leu Tyr		
195	200	205
His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg		
210	215	220
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu		
225	230	235
Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile		
	245	250
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe Ala		
	260	265
Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Pro Met		
	275	280
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser		
290	295	300
Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro		
305	310	315
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn		
	325	330
Ile Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe		
	340	345
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His		
	355	360
His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr		
	370	375
Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu		
385	390	395
Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu		
	405	410
Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu		
	420	425
Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu		
	435	440
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys		
	450	455
Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys		
465	470	475
Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys		
	485	490
Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn		

500	505	510
Arg Glu Lys Ile Asp Gly Ser Glu Ser Gln Val Arg Gln Gln Phe Ser		
515	520	525
Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln		
530	535	540
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser		
545	550	555
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr		
565	570	575
Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro		
580	585	590
Val Gln Leu Thr Ser Ile Ser Cys Pro Glu His Lys Phe Glu Gly Leu		
595	600	605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu		
610	615	620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Ser Lys Asp His Ala		
625	630	635
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu		
645	650	655
Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu		
660	665	670
Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys		
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Ser Arg Lys Ser		
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Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn		

35	40	45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile		
50	55	60
Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly		
65	70	75
Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile		
85	90	95
Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe		
100	105	110
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe		
115	120	125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr		
130	135	140
Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe		
145	150	155
Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn		
165	170	175
Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val Leu		
180	185	190
Trp Gly Val His His Pro Pro Asn Ile Gly Asn Gln Arg Ala Leu Tyr		
195	200	205
His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg		
210	215	220
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu		
225	230	235
Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile		
245	250	255
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe Ala		
260	265	270
Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Pro Met		
275	280	285
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser		
290	295	300
Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro		
305	310	315
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn		
325	330	335
Ile Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe		
340	345	350

Ile	Glu	Gly	Gly	Trp	Thr	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	Tyr	His
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His	Gln	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Gln	Lys	Ser	Thr
370				375				380							
Gln	Asn	Ala	Ile	Asn	Gly	Ile	Thr	Asn	Lys	Val	Asn	Ser	Val	Ile	Glu
385				390				395				400			
Lys	Met	Asn	Thr	Gln	Phe	Thr	Ala	Val	Gly	Lys	Glu	Phe	Asn	Lys	Leu
				405				410				415			
Glu	Arg	Arg	Met	Glu	Asn	Leu	Asn	Lys	Lys	Val	Asp	Asp	Gly	Phe	Leu
				420				425				430			
Asp	Ile	Trp	Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Glu
				435				440				445			
Arg	Thr	Leu	Asp	Phe	His	Asp	Ser	Asn	Val	Lys	Asn	Leu	Tyr	Glu	Lys
				450				455				460			
Val	Lys	Ser	Gln	Leu	Lys	Asn	Asn	Ala	Lys	Glu	Ile	Gly	Asn	Gly	Cys
465				470				475				480			
Phe	Glu	Phe	Tyr	His	Lys	Cys	Asn	Asn	Glu	Cys	Met	Glu	Ser	Val	Lys
				485				490				495			
Asn	Gly	Thr	Tyr	Asp	Tyr	Pro	Lys	Tyr	Ser	Glu	Glu	Ser	Lys	Leu	Asn
				500				505				510			
Arg	Glu	Lys	Ile	Asp	Gly	Ser	Glu	Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser
515				520				525							
Lys	Asp	Ile	Glu	Lys	Leu	Leu	Asn	Glu	Gln	Val	Asn	Lys	Glu	Met	Gln
530				535				540							
Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met	Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser
545				550				555				560			
Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu	Phe	Asp	His	Ala	Ala	Glu	Glu	Tyr
				565				570				575			
Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe	Leu	Asn	Glu	Asn	Asn	Val	Pro
580				585				590							
Val	Gln	Leu	Thr	Ser	Ile	Ser	Ala	Pro	Glu	His	Lys	Phe	Glu	Gly	Leu
595				600				605							
Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr	Glu	His	Glu	Gln	His	Ile	Ser	Glu
610				615				620							
Cys	Ile	Asn	Asn	Ile	Val	Asp	His	Ala	Ile	Lys	Ser	Lys	Asp	His	Ala
625				630				635				640			
Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr	Val	Ala	Glu	Gln	His	Glu	Glu	Glu
				645				650				655			
Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp	Lys	Ile	Glu	Leu	Ile	Gly	Asn	Glu

75

195	200	205
His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg		
210	215	220
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu		
225	230	235
Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile		
245	250	255
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe Ala		
260	265	270
Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Pro Met		
275	280	285
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser		
290	295	300
Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro		
305	310	315
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn		
325	330	335
Ile Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe		
340	345	350
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His		
355	360	365
His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr		
370	375	380
Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu		
385	390	395
Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu		
405	410	415
Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu		
420	425	430
Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu		
435	440	445
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys		
450	455	460
Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys		
465	470	475
Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys		
485	490	495
Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn		
500	505	510

Arg Glu Lys Ile Asp Glu Asn Leu Tyr Phe Gln Gly Ser Glu Ser Gln		
515	520	525
Val Arg Gln Gln Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln		
530	535	540
Val Asn Lys Glu Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser		
545	550	555
Trp Ser Tyr Thr His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp		
565	570	575
His Ala Ala Glu Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu		
580	585	590
Asn Glu Asn Asn Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu		
595	600	605
His Lys Phe Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His		
610	615	620
Glu Gln His Ile Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile		
625	630	635
Lys Cys Lys Asp His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala		
645	650	655
Glu Gln His Glu Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile		
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Glu Leu Ile Gly Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr		
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Val Lys Gly Ile Ala Lys Ser Arg Lys Ser		
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Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn		
35	40	45

Leu	Leu	Glu	Asp	Ser	His	Asn	Gly	Lys	Leu	Cys	Leu	Leu	Lys	Gly	Ile
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Ala	Pro	Leu	Gln	Leu	Gly	Asn	Cys	Ser	Val	Ala	Gly	Trp	Ile	Leu	Gly
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Asn	Pro	Glu	Cys	Glu	Leu	Leu	Ile	Ser	Lys	Glu	Ser	Trp	Ser	Tyr	Ile
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Val	Glu	Thr	Pro	Asn	Pro	Glu	Asn	Gly	Thr	Cys	Phe	Pro	Gly	Tyr	Phe
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Ala	Asp	Tyr	Glu	Glu	Leu	Arg	Glu	Gln	Leu	Ser	Ser	Val	Ser	Ser	Phe
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Glu	Arg	Phe	Glu	Ile	Phe	Pro	Lys	Glu	Ser	Ser	Trp	Pro	Asn	His	Thr
	130					135					140				
Val	Thr	Gly	Val	Ser	Ala	Ser	Cys	Ser	His	Asn	Gly	Lys	Ser	Ser	Phe
145					150					155					160
Tyr	Arg	Asn	Leu	Leu	Trp	Leu	Thr	Gly	Lys	Asn	Gly	Leu	Tyr	Pro	Asn
					165				170					175	
Leu	Ser	Lys	Ser	Tyr	Val	Asn	Asn	Lys	Glu	Lys	Glu	Val	Leu	Val	Leu
				180				185					190		
Trp	Gly	Val	His	His	Pro	Pro	Asn	Ile	Gly	Asn	Gln	Arg	Ala	Leu	Tyr
		195					200					205			
His	Thr	Glu	Asn	Ala	Tyr	Val	Ser	Val	Val	Ser	Ser	His	Tyr	Ser	Arg
		210					215					220			
Arg	Phe	Thr	Pro	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	Arg	Asp	Gln	Glu
225					230					235					240
Gly	Arg	Ile	Asn	Tyr	Tyr	Trp	Thr	Leu	Leu	Glu	Pro	Gly	Asp	Thr	Ile
				245					250					255	
Ile	Phe	Glu	Ala	Asn	Gly	Asn	Leu	Ile	Ala	Pro	Trp	Tyr	Ala	Phe	Ala
			260					265					270		
Leu	Ser	Arg	Gly	Phe	Gly	Ser	Gly	Ile	Ile	Thr	Ser	Asn	Ala	Pro	Met
		275					280					285			
Asp	Glu	Cys	Asp	Ala	Lys	Cys	Gln	Thr	Pro	Gln	Gly	Ala	Ile	Asn	Ser
		290				295					300				
Ser	Leu	Pro	Phe	Gln	Asn	Val	His	Pro	Val	Thr	Ile	Gly	Glu	Cys	Pro
305					310					315					320
Lys	Tyr	Val	Arg	Ser	Ala	Lys	Leu	Arg	Met	Val	Thr	Gly	Leu	Arg	Asn
				325					330					335	
Ile	Pro	Gln	Arg	Glu	Thr	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe
			340					345					350		
Ile	Glu	Gly	Gly	Trp	Thr	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	Tyr	His

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His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr		
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Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu		
385	390	395
Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu		
405	410	415
Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu		
420	425	430
Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu		
435	440	445
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys		
450	455	460
Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys		
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Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys		
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Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn		
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Arg Glu Lys Ile Asp Glu Asn Leu Tyr Phe Gln Gly Ser Glu Ser Gln		
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Val Arg Gln Gln Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln		
530	535	540
Val Asn Lys Glu Met Gln Ser Ser Asn Leu Tyr Met Cys Met Ser Ser		
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Trp Ser Tyr Thr His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp		
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His Ala Ala Glu Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu		
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Asn Glu Asn Asn Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu		
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His Lys Phe Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His		
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Glu Gln His Ile Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile		
625	630	635
Lys Ser Lys Asp His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala		
645	650	655
Glu Gln His Glu Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile		
660	665	670

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 35 40 45
 Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile
 50 55 60
 Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly
 65 70 75 80
 Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile
 85 90 95
 Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe
 100 105 110
 Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
 115 120 125
 Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr
 130 135 140
 Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe
 145 150 155 160
 Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn
 165 170 175
 Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val Leu
 180 185 190
 Trp Gly Val His His Pro Pro Asn Ile Gly Asn Gln Arg Ala Leu Tyr
 195 200 205

His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg			
210	215	220	
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Glu			
225	230	235	240
Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile			
	245	250	255
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe Ala			
	260	265	270
Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Pro Met			
	275	280	285
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser			
290	295	300	
Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro			
305	310	315	320
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn			
	325	330	335
Ile Pro Gln Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe			
	340	345	350
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His			
	355	360	365
His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr			
370	375	380	
Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu			
385	390	395	400
Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu			
	405	410	415
Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu			
	420	425	430
Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu			
435	440	445	
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys			
450	455	460	
Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys			
465	470	475	480
Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys			
	485	490	495
Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn			
	500	505	510
Arg Glu Lys Ile Asp Glu Asn Leu Tyr Phe Gln Gly Ser Glu Ser Gln			

	515					520					525					
Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu	Asn	Glu	Gln	
	530					535					540					
Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met	Ser	Ser	
545	550					555					560					
Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu	Phe	Asp	
	565					570					575					
His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe	Leu	
	580					585					590					
Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Cys	Ile	Ser	Ala	Pro	Glu	
	595					600					605					
His	Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr	Glu	His	
	610					615					620					
Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	His	Ala	Ile	
625	630					635					640					
Lys	Ser	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr	Val	Ala	
	645					650					655					
Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp	Lys	Ile	
	660					665					670					
Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	Asp	Gln	Tyr	
	675					680					685					
Val	Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser							
	690					695										

$\langle 210 \rangle$ 12

<211> 698

<212> PRT

〈213〉 人工序列

 $\langle 220 \rangle$

〈221〉 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 12

Met	Lys	Ala	Lys	Leu	Leu	Val	Leu	Leu	Cys	Thr	Phe	Thr	Ala	Thr	Tyr
1				5					10					15	
Ala	Asp	Thr	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Asp	Thr
			20					25					30		
Val	Asp	Thr	Val	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Asn
		35					40					45			
Leu	Leu	Glu	Asp	Ser	His	Asn	Gly	Lys	Leu	Cys	Leu	Leu	Lys	Gly	Ile

50	55	60
Ala Pro Leu Gln Leu Gly Asn Cys Ser Val	Ala Gly Trp Ile Leu Gly	
65	70	75
Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys	Glu Ser Trp Ser Tyr Ile	80
85	90	95
Val Glu Thr Pro Asn Pro Glu Asn Gly Thr	Cys Phe Pro Gly Tyr Phe	
100	105	110
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu	Ser Ser Val Ser Ser Phe	
115	120	125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser	Ser Trp Pro Asn His Thr	
130	135	140
Val Thr Gly Val Ser Ala Ser Cys Ser His	Asn Gly Lys Ser Ser Phe	
145	150	155
Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys	Asn Gly Leu Tyr Pro Asn	
165	170	175
Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu	Lys Glu Val Leu Val Leu	
180	185	190
Trp Gly Val His His Pro Pro Asn Ile Gly	Asn Gln Arg Ala Leu Tyr	
195	200	205
His Thr Glu Asn Ala Tyr Val Ser Val Val	Ser Ser His Tyr Ser Arg	
210	215	220
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro	Lys Val Arg Asp Gln Glu	
225	230	235
Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu	Glu Pro Gly Asp Thr Ile	
245	250	255
Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala	Pro Trp Tyr Ala Phe Ala	
260	265	270
Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile	Thr Ser Asn Ala Pro Met	
275	280	285
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro	Gln Gly Ala Ile Asn Ser	
290	295	300
Ser Leu Pro Phe Gln Asn Val His Pro Val	Thr Ile Gly Glu Cys Pro	
305	310	315
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met	Val Thr Gly Leu Arg Asn	
325	330	335
Ile Pro Gln Arg Glu Thr Arg Gly Leu Phe	Gly Ala Ile Ala Gly Phe	
340	345	350
Ile Glu Gly Gly Trp Thr Gly Met Val Asp	Gly Trp Tyr Gly Tyr His	
355	360	365

His	Gln	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Gln	Lys	Ser	Thr	
370						375						380				
Gln	Asn	Ala	Ile	Asn	Gly	Ile	Thr	Asn	Lys	Val	Asn	Ser	Val	Ile	Glu	
385						390						395			400	
Lys	Met	Asn	Thr	Gln	Phe	Thr	Ala	Val	Gly	Lys	Glu	Phe	Asn	Lys	Leu	
			405						410						415	
Glu	Arg	Arg	Met	Glu	Asn	Leu	Asn	Lys	Lys	Val	Asp	Asp	Gly	Phe	Leu	
			420						425						430	
Asp	Ile	Trp	Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn	Glu	
435						440						445				
Arg	Thr	Leu	Asp	Phe	His	Asp	Ser	Asn	Val	Lys	Asn	Leu	Tyr	Glu	Lys	
450						455						460				
Val	Lys	Ser	Gln	Leu	Lys	Asn	Asn	Ala	Lys	Glu	Ile	Gly	Asn	Gly	Cys	
465						470						475			480	
Phe	Glu	Phe	Tyr	His	Lys	Cys	Asn	Asn	Glu	Cys	Met	Glu	Ser	Val	Lys	
			485						490						495	
Asn	Gly	Thr	Tyr	Asp	Tyr	Pro	Lys	Tyr	Ser	Glu	Glu	Ser	Lys	Leu	Asn	
			500						505						510	
Arg	Glu	Lys	Ile	Asp	Glu	Asn	Leu	Tyr	Phe	Gln	Gly	Ser	Glu	Ser	Gln	
515						520						525				
Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu	Asn	Glu	Gln	
530						535						540				
Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met	Ser	Ser	
545						550						555			560	
Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu	Phe	Asp	
			565						570						575	
His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe	Leu	
			580						585						590	
Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	Cys	Pro	Glu	
595						600						605				
His	Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr	Glu	His	
610						615						620				
Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	His	Ala	Ile	
625						630						635			640	
Lys	Ser	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr	Val	Ala	
			645						650						655	
Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp	Lys	Ile	
			660						665						670	
Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	Asp	Gln	Tyr	

675	680	685
Val Lys Gly Ile Ala Lys Ser Arg Lys Ser		
690	695	
<210> 13		
<211> 698		
<212> PRT		
<213> 人工序列		
<220>		
<221> 来源		
<223> /注释="人工序列的描述：合成多肽"		
<400> 13		
Met Lys Ala Lys Leu Leu Val Leu Leu Cys Thr Phe Thr Ala Thr Tyr		
1 5 10 15		
Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr		
20 25 30		
Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn		
35 40 45		
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Leu Leu Lys Gly Ile		
50 55 60		
Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly		
65 70 75 80		
Asn Pro Glu Cys Glu Leu Leu Ile Ser Lys Glu Ser Trp Ser Tyr Ile		
85 90 95		
Val Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe		
100 105 110		
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe		
115 120 125		
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr		
130 135 140		
Val Thr Gly Val Ser Ala Ser Cys Ser His Asn Gly Lys Ser Ser Phe		
145 150 155 160		
Tyr Arg Asn Leu Leu Trp Leu Thr Gly Lys Asn Gly Leu Tyr Pro Asn		
165 170 175		
Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val Leu		
180 185 190		
Trp Gly Val His His Pro Pro Asn Ile Gly Asn Gln Arg Ala Leu Tyr		
195 200 205		
His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser Arg		

210	215	220
Arg Phe Thr Pro Glu Ile	Ala Lys Arg Pro Lys Val	Arg Asp Gln Glu
225	230	235
Gly Arg Ile Asn Tyr Tyr	Trp Thr Leu Leu Glu Pro	Gly Asp Thr Ile
245	250	255
Ile Phe Glu Ala Asn Gly	Asn Leu Ile Ala Pro	Trp Tyr Ala Phe Ala
260	265	270
Leu Ser Arg Gly Phe Gly	Ser Gly Ile Ile Thr	Ser Asn Ala Pro Met
275	280	285
Asp Glu Cys Asp Ala Lys	Cys Gln Thr Pro Gln	Gly Ala Ile Asn Ser
290	295	300
Ser Leu Pro Phe Gln Asn	Val His Pro Val Thr	Ile Gly Glu Cys Pro
305	310	315
Lys Tyr Val Arg Ser Ala	Lys Leu Arg Met Val	Thr Gly Leu Arg Asn
325	330	335
Ile Pro Gln Arg Glu Thr	Arg Gly Leu Phe Gly	Ala Ile Ala Gly Phe
340	345	350
Ile Glu Gly Gly Trp Thr	Gly Met Val Asp Gly	Trp Tyr Gly Tyr His
355	360	365
His Gln Asn Glu Gln Gly	Ser Gly Tyr Ala Ala	Asp Gln Lys Ser Thr
370	375	380
Gln Asn Ala Ile Asn Gly	Ile Thr Asn Lys Val	Asn Ser Val Ile Glu
385	390	395
Lys Met Asn Thr Gln Phe	Thr Ala Val Gly Lys	Glu Phe Asn Lys Leu
405	410	415
Glu Arg Arg Met Glu Asn	Leu Asn Lys Lys Val	Asp Asp Gly Phe Leu
420	425	430
Asp Ile Trp Thr Tyr Asn	Ala Glu Leu Leu Val	Leu Leu Glu Asn Glu
435	440	445
Arg Thr Leu Asp Phe His	Asp Ser Asn Val Lys	Asn Leu Tyr Glu Lys
450	455	460
Val Lys Ser Gln Leu Lys	Asn Asn Ala Lys Glu	Ile Gly Asn Gly Cys
465	470	475
Phe Glu Phe Tyr His Lys	Cys Asn Asn Glu Cys	Met Glu Ser Val Lys
485	490	495
Asn Gly Thr Tyr Asp Tyr	Pro Lys Tyr Ser Glu	Glu Ser Lys Leu Asn
500	505	510
Arg Glu Lys Ile Asp Glu	Asn Leu Tyr Phe Gln	Gly Ser Glu Ser Gln
515	520	525

Val Arg Gln Gln Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln
 530 535 540
 Val Asn Lys Glu Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser
 545 550 555 560
 Trp Ser Tyr Thr His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp
 565 570 575
 His Ala Ala Glu Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu
 580 585 590
 Asn Glu Asn Asn Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu
 595 600 605
 His Lys Phe Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His
 610 615 620
 Glu Gln His Ile Ser Glu Cys Ile Asn Asn Ile Val Asp His Ala Ile
 625 630 635 640
 Lys Ser Lys Asp His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala
 645 650 655
 Glu Gln His Glu Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile
 660 665 670
 Glu Leu Ile Gly Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr
 675 680 685
 Val Lys Gly Ile Ala Lys Ser Arg Lys Ser
 690 695

<210> 14

<211> 694

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 14

Met Glu Ala Arg Leu Leu Val Leu Leu Cys Ala Phe Ala Ala Thr Asn
 1 5 10 15
 Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
 20 25 30
 Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
 35 40 45
 Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Lys Leu Lys Gly Ile
 50 55 60

Ala Pro Leu Gln Leu Gly Lys Cys Asn Ile Ala Gly Trp Leu Leu Gly			
65	70	75	80
Asn Pro Glu Cys Asp Leu Leu Leu Thr Ala Ser Ser Trp Ser Tyr Ile			
	85	90	95
Val Glu Thr Ser Asn Ser Glu Asn Gly Thr Cys Phe Pro Gly Asp Phe			
	100	105	110
Ile Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe			
	115	120	125
Glu Lys Phe Glu Ile Phe Pro Lys Thr Ser Ser Trp Pro Asn His Glu			
	130	135	140
Thr Thr Lys Gly Val Thr Ala Ala Cys Ser Tyr Ala Gly Ala Ser Ser			
145	150	155	160
Phe Tyr Arg Asn Leu Leu Trp Leu Thr Lys Lys Gly Ser Ser Tyr Pro			
	165	170	175
Lys Leu Ser Lys Ser Tyr Val Asn Asn Lys Gly Lys Glu Val Leu Val			
	180	185	190
Leu Trp Gly Val His His Pro Pro Thr Gly Thr Asp Gln Gln Ser Leu			
	195	200	205
Tyr Gln Asn Ala Asp Ala Tyr Val Ser Val Gly Ser Ser Lys Tyr Asn			
	210	215	220
Arg Arg Phe Thr Pro Glu Ile Ala Ala Arg Pro Lys Val Arg Asp Gln			
225	230	235	240
Ala Gly Arg Met Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr			
	245	250	255
Ile Thr Phe Glu Ala Thr Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe			
	260	265	270
Ala Leu Asn Arg Gly Ser Gly Ser Gly Ile Ile Thr Ser Asp Ala Pro			
	275	280	285
Val His Asp Cys Asn Thr Lys Cys Gln Thr Pro His Gly Ala Ile Asn			
	290	295	300
Ser Ser Leu Pro Phe Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys			
305	310	315	320
Pro Lys Tyr Val Arg Ser Thr Lys Leu Arg Met Ala Thr Gly Leu Arg			
	325	330	335
Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly			
	340	345	350
Phe Ile Glu Gly Gly Trp Thr Gly Met Ile Asp Gly Trp Tyr Gly Tyr			
	355	360	365
His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser			

370	375	380
Thr Gln Asn Ala Ile Asp Gly Ile Thr Asn Lys Val Asn Ser Val Ile		
385	390	395
Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Asn		400
	405	410
Leu Glu Arg Arg Ile Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe		415
	420	425
Leu Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn		430
	435	440
Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Arg Asn Leu Tyr Glu		445
	450	455
Lys Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly		460
465	470	475
Cys Phe Glu Phe Tyr His Lys Cys Asp Asp Ala Cys Met Glu Ser Val		480
	485	490
Arg Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu		495
	500	505
Asn Arg Glu Glu Ile Asp Ser Gly Gly Glu Ser Gln Val Arg Gln Gln		510
	515	520
Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu		525
	530	535
Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr		540
545	550	555
His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu		560
	565	570
Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn		575
	580	585
Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu		590
	595	600
Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile		605
	610	615
Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp		620
625	630	635
His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu		640
	645	650
Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly		655
	660	665
Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile		670
	675	680
		685

Ala Lys Ser Arg Lys Ser

690

<210> 15

<211> 694

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 15

Met Lys Ala Ile Leu Val Val Leu Leu Tyr Thr Phe Ala Thr Ala Asn

1 5 10 15

Ala Asp Thr Leu Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr

20 25 30

Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn

35 40 45

Leu Leu Glu Asp Lys His Asn Gly Lys Leu Cys Lys Leu Arg Gly Val

50 55 60

Ala Pro Leu His Leu Gly Lys Cys Asn Ile Ala Gly Trp Ile Leu Gly

65 70 75 80

Asn Pro Glu Cys Glu Ser Leu Ser Thr Ala Ser Ser Trp Ser Tyr Ile

85 90 95

Val Glu Thr Pro Ser Ser Asp Asn Gly Thr Cys Phe Pro Gly Asp Phe

100 105 110

Ile Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe

115 120 125

Glu Arg Phe Glu Ile Phe Pro Lys Thr Ser Ser Trp Pro Asn His Asp

130 135 140

Ser Asn Lys Gly Val Thr Ala Ala Cys Pro His Ala Gly Ala Lys Ser

145 150 155 160

Phe Tyr Lys Asn Leu Ile Trp Leu Val Lys Lys Gly Asn Ser Tyr Pro

165 170 175

Lys Leu Ser Lys Ser Tyr Ile Asn Asp Lys Gly Lys Glu Val Leu Val

180 185 190

Leu Trp Gly Ile His His Pro Ser Thr Ser Ala Asp Gln Gln Ser Leu

195 200 205

Tyr Gln Asn Ala Asp Thr Tyr Val Phe Val Gly Ser Ser Arg Tyr Ser

210 215 220

Lys	Lys	Phe	Lys	Pro	Glu	Ile	Ala	Ile	Arg	Pro	Lys	Val	Arg	Asp	Gln
225					230				235						240
Glu	Gly	Arg	Met	Asn	Tyr	Tyr	Trp	Thr	Leu	Val	Glu	Pro	Gly	Asp	Lys
			245						250						255
Ile	Thr	Phe	Glu	Ala	Thr	Gly	Asn	Leu	Val	Val	Pro	Arg	Tyr	Ala	Phe
			260						265						270
Ala	Met	Glu	Arg	Asn	Ala	Gly	Ser	Gly	Ile	Ile	Ile	Ser	Asp	Thr	Pro
			275						280						285
Val	His	Asp	Cys	Asn	Thr	Thr	Cys	Gln	Thr	Pro	Lys	Gly	Ala	Ile	Asn
			290						295						300
Thr	Ser	Leu	Pro	Phe	Gln	Asn	Ile	His	Pro	Ile	Thr	Ile	Gly	Lys	Cys
305					310										320
Pro	Lys	Tyr	Val	Lys	Ser	Thr	Lys	Leu	Arg	Leu	Ala	Thr	Gly	Leu	Arg
					325										335
Asn	Ile	Pro	Ser	Ile	Gln	Ser	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly
			340												350
Phe	Ile	Glu	Gly	Gly	Trp	Thr	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	Tyr
			355												365
His	His	Gln	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Leu	Lys	Ser
			370												380
Thr	Gln	Asn	Ala	Ile	Asp	Glu	Ile	Thr	Asn	Lys	Val	Asn	Ser	Val	Ile
385					390										400
Glu	Lys	Met	Asn	Thr	Gln	Phe	Thr	Ala	Val	Gly	Lys	Glu	Phe	Asn	His
					405										415
Leu	Glu	Lys	Arg	Ile	Glu	Asn	Leu	Asn	Lys	Lys	Val	Asp	Asp	Gly	Phe
			420												430
Leu	Asp	Ile	Trp	Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn
			435												445
Glu	Arg	Thr	Leu	Asp	Tyr	His	Asp	Ser	Asn	Val	Lys	Asn	Leu	Tyr	Glu
			450												460
Lys	Val	Arg	Ser	Gln	Leu	Lys	Asn	Asn	Ala	Lys	Glu	Ile	Gly	Asn	Gly
465					470										480
Cys	Phe	Glu	Phe	Tyr	His	Lys	Cys	Asp	Asn	Thr	Cys	Met	Glu	Ser	Val
					485										495
Lys	Asn	Gly	Thr	Tyr	Asp	Tyr	Pro	Lys	Tyr	Ser	Glu	Glu	Ala	Lys	Leu
			500												510
Asn	Arg	Glu	Glu	Ile	Asp	Ser	Gly	Gly	Glu	Ser	Gln	Val	Arg	Gln	Gln
			515												525
Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu	Asn	Glu	Gln	Val	Asn	Lys	Glu

530	535	540
Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr		
545	550	555
His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu		
565	570	575
Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn		
580	585	590
Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu		
595	600	605
Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile		
610	615	620
Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp		
625	630	635
His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu		
645	650	655
Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly		
660	665	670
Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile		
675	680	685
Ala Lys Ser Arg Lys Ser		
690		
<210> 16		
<211> 694		
<212> PRT		
<213> 人工序列		
<220>		
<221> 来源		
<223> /注释="人工序列的描述：合成多肽"		
<400> 16		
Met Lys Ala Arg Leu Leu Ile Leu Leu Cys Ala Leu Ser Ala Thr Asp		
1	5	10
Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr		
20	25	30
Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn		
35	40	45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Arg Leu Lys Gly Ile		
50	55	60
Ala Pro Leu Gln Leu Gly Lys Cys Asn Ile Ala Gly Trp Ile Leu Gly		

65	70	75	80
Asn Pro Glu Cys Glu Ser Leu Leu Ser Asn Arg Ser Trp Ser Tyr Ile			
	85	90	95
Ala Glu Thr Pro Asn Ser Glu Asn Gly Ile Cys Phe Pro Gly Asp Phe			
	100	105	110
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe			
	115	120	125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Lys His Asn			
	130	135	140
Ile Thr Arg Gly Val Thr Val Ala Cys Ser His Ala Lys Lys Ser Ser			
145	150	155	160
Phe Tyr Lys Asn Leu Leu Trp Leu Thr Glu Ala Asn Gly Leu Tyr Pro			
	165	170	175
Ser Leu Ser Lys Ser Tyr Val Asn Asp Arg Glu Lys Glu Val Leu Val			
	180	185	190
Leu Trp Gly Val His His Pro Ser Asn Ile Glu Asp Gln Arg Thr Leu			
	195	200	205
Tyr Arg Lys Glu Asn Ala Tyr Val Ser Val Val Ser Ser Asn Tyr Asn			
	210	215	220
Arg Arg Phe Thr Pro Glu Ile Ala Glu Arg Pro Lys Val Arg Gly Gln			
225	230	235	240
Pro Gly Arg Met Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Lys			
	245	250	255
Ile Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe			
	260	265	270
Ala Leu Ser Arg Gly Pro Gly Ser Gly Ile Ile Thr Ser Asn Ala Ser			
	275	280	285
Met Asp Glu Cys Asp Thr Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn			
	290	295	300
Ser Ser Leu Pro Phe Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys			
305	310	315	320
Pro Lys Tyr Val Arg Ser Thr Lys Leu Arg Met Val Thr Gly Leu Arg			
	325	330	335
Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly			
	340	345	350
Phe Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr			
	355	360	365
His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser			
	370	375	380

Thr	Gln	Asn	Ala	Ile	Asn	Gly	Ile	Thr	Asn	Lys	Val	Asn	Ser	Val	Ile
385					390					395					400
Glu	Lys	Met	Asn	Thr	Gln	Phe	Thr	Ala	Val	Gly	Lys	Glu	Phe	Asn	Lys
			405						410					415	
Leu	Glu	Lys	Arg	Met	Glu	Asn	Leu	Asn	Lys	Lys	Val	Asp	Asp	Gly	Phe
			420					425					430		
Leu	Asp	Ile	Trp	Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu	Leu	Glu	Asn
		435					440						445		
Glu	Arg	Thr	Leu	Asp	Phe	His	Asp	Ser	Asn	Val	Lys	Asn	Leu	Tyr	Glu
	450					455					460				
Lys	Val	Lys	Asn	Gln	Leu	Arg	Asn	Asn	Ala	Lys	Glu	Ile	Gly	Asn	Gly
465				470						475					480
Cys	Phe	Glu	Phe	Tyr	His	Lys	Cys	Asp	Asn	Glu	Cys	Met	Glu	Ser	Val
			485					490						495	
Lys	Asn	Gly	Thr	Tyr	Asp	Tyr	Pro	Lys	Tyr	Ser	Glu	Glu	Ser	Lys	Leu
		500						505					510		
Asn	Arg	Ala	Lys	Ile	Asp	Ser	Gly	Gly	Glu	Ser	Gln	Val	Arg	Gln	Gln
		515					520					525			
Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu	Asn	Glu	Gln	Val	Asn	Lys	Glu
	530					535					540				
Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met	Ser	Ser	Trp	Ser	Tyr	Thr
545				550						555					560
His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu	Phe	Asp	His	Ala	Ala	Glu
			565					570					575		
Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe	Leu	Asn	Glu	Asn	Asn
		580						585					590		
Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	Ala	Pro	Glu	His	Lys	Phe	Glu
		595					600						605		
Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr	Glu	His	Glu	Gln	His	Ile
	610					615				620					
Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	His	Ala	Ile	Lys	Cys	Lys	Asp
625				630						635					640
His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr	Val	Ala	Glu	Gln	His	Glu
			645					650					655		
Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp	Lys	Ile	Glu	Leu	Ile	Gly
		660						665					670		
Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	Asp	Gln	Tyr	Val	Lys	Gly	Ile
		675					680						685		
Ala	Lys	Ser	Arg	Lys	Ser										

95

225	230	235	240
Ala Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr			
	245	250	255
Ile Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe			
	260	265	270
Ala Leu Ser Arg Asp Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Ser			
	275	280	285
Met Asp Glu Cys Asp Thr Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn			
	290	295	300
Ser Ser Leu Pro Phe Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys			
305	310	315	320
Pro Lys Tyr Val Lys Ser Thr Lys Leu Arg Met Val Thr Gly Leu Arg			
	325	330	335
Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly			
	340	345	350
Phe Ile Glu Gly Gly Trp Thr Gly Met Ile Asp Gly Trp Tyr Gly Tyr			
	355	360	365
His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser			
	370	375	380
Thr Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile			
385	390	395	400
Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys			
	405	410	415
Leu Glu Lys Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe			
	420	425	430
Leu Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn			
	435	440	445
Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu			
	450	455	460
Lys Val Lys Asn Gln Leu Arg Asn Asn Ala Lys Glu Ile Gly Asn Gly			
465	470	475	480
Cys Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val			
	485	490	495
Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu			
	500	505	510
Asn Arg Glu Lys Ile Asp Ser Gly Gly Glu Ser Gln Val Arg Gln Gln			
	515	520	525
Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu			
	530	535	540

Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met	Ser	Ser	Trp	Ser	Tyr	Thr
545					550					555					560
His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu	Phe	Asp	His	Ala	Ala	Glu
				565						570					575
Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe	Leu	Asn	Glu	Asn	Asn
				580						585					590
Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	Ala	Pro	Glu	His	Lys	Phe	Glu
				595						600					605
Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr	Glu	His	Glu	Gln	His	Ile
				610											620
Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	His	Ala	Ile	Lys	Cys	Lys	Asp
625					630					635					640
His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr	Val	Ala	Glu	Gln	His	Glu
				645						650					655
Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp	Lys	Ile	Glu	Leu	Ile	Gly
				660						665					670
Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	Asp	Gln	Tyr	Val	Lys	Gly	Ile
				675						680					685
Ala	Lys	Ser	Arg	Lys	Ser										
				690											

<210> 18

<211> 694

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 18

Met	Lys	Ala	Lys	Leu	Leu	Val	Leu	Leu	Cys	Ala	Leu	Ser	Ala	Thr	Asp
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Ala	Asp	Thr	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Asp	Thr
				20					25					30	
Val	Asp	Thr	Val	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Asn
				35					40					45	
Leu	Leu	Glu	Asp	Ser	His	Asn	Gly	Lys	Leu	Cys	Arg	Leu	Lys	Gly	Ile
				50					55					60	
Ala	Pro	Leu	Gln	Leu	Gly	Lys	Cys	Ser	Ile	Ala	Gly	Trp	Ile	Leu	Gly
65					70					75					80

Asn	Pro	Glu	Cys	Glu	Ser	Leu	Phe	Ser	Lys	Lys	Ser	Trp	Ser	Tyr	Ile	
				85					90					95		
Ala	Glu	Thr	Pro	Asn	Ser	Glu	Asn	Gly	Thr	Cys	Phe	Pro	Gly	Tyr	Phe	
			100					105					110			
Ala	Asp	Tyr	Glu	Glu	Leu	Arg	Glu	Gln	Leu	Ser	Ser	Val	Ser	Ser	Phe	
		115					120					125				
Glu	Arg	Phe	Glu	Ile	Phe	Pro	Lys	Glu	Arg	Ser	Trp	Pro	Lys	His	Asn	
	130					135					140					
Val	Thr	Arg	Gly	Val	Thr	Ala	Ser	Cys	Ser	His	Lys	Gly	Lys	Ser	Ser	
145					150					155					160	
Phe	Tyr	Arg	Asn	Leu	Leu	Trp	Leu	Thr	Glu	Lys	Asn	Gly	Ser	Tyr	Pro	
			165					170						175		
Asn	Leu	Ser	Lys	Ser	Tyr	Val	Asn	Asn	Lys	Glu	Lys	Glu	Val	Leu	Val	
		180					185					190				
Leu	Trp	Gly	Val	His	His	Pro	Ser	Asn	Ile	Glu	Asp	Gln	Lys	Thr	Ile	
	195					200						205				
Tyr	Arg	Lys	Glu	Asn	Ala	Tyr	Val	Ser	Val	Val	Ser	Ser	Asn	Tyr	Asn	
	210				215						220					
Arg	Arg	Phe	Thr	Pro	Glu	Ile	Ala	Glu	Arg	Pro	Lys	Val	Arg	Gly	Gln	
225				230					235					240		
Ala	Gly	Arg	Ile	Asn	Tyr	Tyr	Trp	Thr	Leu	Leu	Glu	Pro	Gly	Asp	Thr	
			245					250					255			
Ile	Ile	Phe	Glu	Ala	Asn	Gly	Asn	Leu	Ile	Ala	Pro	Trp	Tyr	Ala	Phe	
		260				265						270				
Ala	Leu	Ser	Arg	Gly	Phe	Gly	Ser	Gly	Ile	Ile	Thr	Ser	Asn	Ala	Ser	
	275					280					285					
Met	Asp	Glu	Cys	Asp	Thr	Lys	Cys	Gln	Thr	Pro	Gln	Gly	Ala	Ile	Asn	
	290				295						300					
Ser	Ser	Leu	Pro	Phe	Gln	Asn	Val	His	Pro	Val	Thr	Ile	Gly	Glu	Cys	
305			310					315						320		
Pro	Lys	Tyr	Val	Arg	Ser	Thr	Lys	Leu	Arg	Met	Val	Thr	Gly	Leu	Arg	
			325					330					335			
Asn	Ile	Pro	Ser	Ile	Gln	Ser	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	
		340				345						350				
Phe	Ile	Glu	Gly	Gly	Trp	Thr	Gly	Met	Ile	Asp	Gly	Trp	Tyr	Gly	Tyr	
	355					360					365					
His	His	Gln	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Gln	Lys	Ser	
	370					375					380					
Thr	Gln	Asn	Ala	Ile	Asn	Gly	Ile	Thr	Asn	Lys	Val	Asn	Ser	Val	Ile	

385	390	395	400
Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys			
	405	410	415
Leu Glu Lys Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe			
	420	425	430
Leu Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn			
	435	440	445
Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu			
	450	455	460
Lys Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly			
465	470	475	480
Cys Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val			
	485	490	495
Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu			
	500	505	510
Asn Arg Glu Lys Ile Asp Ser Gly Gly Glu Ser Gln Val Arg Gln Gln			
	515	520	525
Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu			
	530	535	540
Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr			
545	550	555	560
His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu			
	565	570	575
Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn			
	580	585	590
Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu			
	595	600	605
Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile			
	610	615	620
Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp			
625	630	635	640
His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu			
	645	650	655
Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly			
	660	665	670
Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile			
	675	680	685
Ala Lys Ser Arg Lys Ser			
690			

<210> 19

<211> 690

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 19

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Met Ala Ile Ile Tyr Leu Ile Leu Leu Phe Thr Ala Val Arg Gly Asp
1           5           10           15
Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Lys Val Asp
          20           25           30
Thr Ile Leu Glu Arg Asn Val Thr Val Thr His Ala Lys Asp Ile Leu
          35           40           45
Glu Lys Thr His Asn Gly Lys Leu Cys Lys Leu Asn Gly Ile Pro Pro
          50           55           60
Leu Glu Leu Gly Asp Cys Ser Ile Ala Gly Trp Leu Leu Gly Asn Pro
65           70           75           80
Glu Cys Asp Arg Leu Leu Ser Val Pro Glu Trp Ser Tyr Ile Met Glu
          85           90           95
Lys Glu Asn Pro Arg Asp Gly Leu Cys Phe Pro Gly Ser Phe Asn Asp
          100          105          110
Tyr Glu Glu Leu Lys His Leu Leu Ser Ser Val Lys His Phe Glu Lys
          115          120          125
Val Lys Ile Leu Pro Lys Asp Arg Trp Thr Gln His Thr Thr Thr Gly
          130          135          140
Gly Ser Arg Ala Cys Ala Val Ser Gly Asn Pro Ser Phe Phe Arg Asn
145          150          155          160
Met Val Trp Leu Thr Lys Lys Glu Ser Asn Tyr Pro Val Ala Lys Gly
          165          170          175
Ser Tyr Asn Asn Thr Ser Gly Glu Gln Met Leu Ile Ile Trp Gly Val
          180          185          190
His His Pro Asn Asp Glu Thr Glu Gln Arg Thr Leu Tyr Gln Asn Val
          195          200          205
Gly Thr Tyr Val Ser Val Gly Thr Ser Thr Leu Asn Lys Arg Ser Thr
          210          215          220
Pro Asp Ile Ala Thr Arg Pro Lys Val Asn Gly Leu Gly Ser Arg Met
225          230          235          240

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Glu Phe Ser Trp Thr Leu Leu Asp Met Trp Asp Thr Ile Asn Phe Glu		
245	250	255
Ser Thr Gly Asn Leu Ile Ala Pro Glu Tyr Gly Phe Lys Ile Ser Lys		
260	265	270
Arg Gly Ser Ser Gly Ile Met Lys Thr Glu Gly Thr Leu Glu Asn Cys		
275	280	285
Glu Thr Lys Cys Gln Thr Pro Leu Gly Ala Ile Asn Thr Thr Leu Pro		
290	295	300
Phe His Asn Val His Pro Leu Thr Ile Gly Glu Cys Pro Lys Tyr Val		
305	310	315
Lys Ser Glu Lys Leu Val Leu Ala Thr Gly Leu Arg Asn Val Pro Gln		
325	330	335
Ile Glu Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly		
340	345	350
Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His Ser Asn		
355	360	365
Asp Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln Lys Ala		
370	375	380
Phe Asp Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu Lys Met Asn		
385	390	395
Thr Gln Phe Glu Ala Val Gly Lys Glu Phe Ser Asn Leu Glu Arg Arg		
405	410	415
Leu Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp Val Trp		
420	425	430
Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg Thr Leu		
435	440	445
Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val Arg Met		
450	455	460
Gln Leu Arg Asp Asn Val Lys Glu Leu Gly Asn Gly Cys Phe Glu Phe		
465	470	475
Tyr His Lys Cys Asp Asp Glu Cys Met Asn Ser Val Lys Asn Gly Thr		
485	490	495
Tyr Asp Tyr Pro Lys Tyr Glu Glu Glu Ser Lys Leu Asn Arg Asn Glu		
500	505	510
Ile Lys Ser Gly Gly Glu Ser Gln Val Arg Gln Gln Phe Ser Lys Asp		
515	520	525
Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln Ser Ser		
530	535	540
Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser Leu Asp		

545	550	555	560
Gly Ala Gly Leu Phe	Leu Phe Asp His	Ala Ala Glu Glu Tyr	Glu His
565	570	575	
Ala Lys Lys Leu Ile	Ile Phe Leu Asn	Glu Asn Asn Val	Pro Val Gln
580	585	590	
Leu Thr Ser Ile Ser	Ala Pro Glu His	Lys Phe Glu Gly	Leu Thr Gln
595	600	605	
Ile Phe Gln Lys Ala	Tyr Glu His Glu	Gln His Ile Ser	Glu Ser Ile
610	615	620	
Asn Asn Ile Val Asp	His Ala Ile Lys	Cys Lys Asp His	Ala Thr Phe
625	630	635	640
Asn Phe Leu Gln Trp	Tyr Val Ala Glu	Gln His Glu Glu	Glu Val Leu
645	650	655	
Phe Lys Asp Ile Leu	Asp Lys Ile Glu	Leu Ile Gly Asn	Glu Asn His
660	665	670	
Gly Leu Tyr Leu Ala	Asp Gln Tyr Val	Lys Gly Ile Ala	Lys Ser Arg
675	680	685	
Lys Ser			
690			
<210> 20			
<211> 692			
<212> PRT			
<213> 人工序列			
<220>			
<221> 来源			
<223> /注释="人工序列的描述：合成多肽"			
<400> 20			
Met Glu Lys Ile Val	Leu Leu Leu Ala	Ile Val Ser Leu	Val Lys Ser
1	5	10	15
Asp Gln Ile Cys Ile	Gly Tyr His Ala	Asn Asn Ser Thr	Glu Gln Val
20	25	30	
Asp Thr Ile Met Glu	Lys Asn Val Thr	Val Thr His Ala	Gln Asp Ile
35	40	45	
Leu Glu Lys Thr His	Asn Gly Lys Leu	Cys Asp Leu Asp	Gly Val Lys
50	55	60	
Pro Leu Ile Leu Arg	Asp Cys Ser Val	Ala Gly Trp Leu	Leu Gly Asn
65	70	75	80
Pro Met Cys Asp Glu	Phe Ile Asn Val	Pro Glu Trp Ser	Tyr Ile Val

				85					90					95			
Glu	Lys	Ala	Asn	Pro	Thr	Asn	Asp	Leu	Cys	Phe	Pro	Gly	Ser	Phe	Asn		
				100					105					110			
Asp	Tyr	Glu	Glu	Leu	Lys	His	Leu	Leu	Ser	Arg	Ile	Asn	His	Phe	Glu		
				115					120					125			
Lys	Ile	Gln	Ile	Ile	Pro	Lys	Ser	Ser	Trp	Ser	Asp	His	Glu	Ala	Ser		
				130					135					140			
Ser	Gly	Val	Ser	Ser	Ala	Cys	Pro	Tyr	Leu	Gly	Ser	Pro	Ser	Phe	Phe		
				145					150					155			160
Arg	Asn	Val	Val	Trp	Leu	Ile	Lys	Lys	Asn	Ser	Thr	Tyr	Pro	Thr	Ile		
				165					170					175			
Lys	Lys	Ser	Tyr	Asn	Asn	Thr	Asn	Gln	Glu	Asp	Leu	Leu	Val	Leu	Trp		
				180					185					190			
Gly	Ile	His	His	Pro	Asn	Asp	Ala	Ala	Glu	Gln	Thr	Arg	Leu	Tyr	Gln		
				195					200					205			
Asn	Pro	Thr	Thr	Tyr	Ile	Ser	Ile	Gly	Thr	Ser	Thr	Leu	Asn	Gln	Arg		
				210					215					220			
Leu	Val	Pro	Lys	Ile	Ala	Thr	Arg	Ser	Lys	Val	Asn	Gly	Gln	Ser	Gly		
				225					230					235			240
Arg	Met	Glu	Phe	Phe	Trp	Thr	Ile	Leu	Lys	Pro	Asn	Asp	Ala	Ile	Asn		
				245					250					255			
Phe	Glu	Ser	Asn	Gly	Asn	Phe	Ile	Ala	Pro	Glu	Tyr	Ala	Tyr	Lys	Ile		
				260					265					270			
Val	Lys	Lys	Gly	Asp	Ser	Ala	Ile	Met	Lys	Ser	Glu	Leu	Glu	Tyr	Gly		
				275					280					285			
Asn	Cys	Asn	Thr	Lys	Cys	Gln	Thr	Pro	Met	Gly	Ala	Ile	Asn	Ser	Ser		
				290					295					300			
Met	Pro	Phe	His	Asn	Ile	His	Pro	Leu	Thr	Ile	Gly	Glu	Cys	Pro	Lys		
				305					310					315			320
Tyr	Val	Lys	Ser	Asn	Arg	Leu	Val	Leu	Ala	Thr	Gly	Leu	Arg	Asn	Ser		
				325					330					335			
Pro	Gln	Arg	Glu	Thr	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile		
				340					345					350			
Glu	Gly	Gly	Trp	Gln	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	Tyr	His	His		
				355					360					365			
Ser	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Lys	Glu	Ser	Thr	Gln		
				370					375					380			
Lys	Ala	Ile	Asp	Gly	Val	Thr	Asn	Lys	Val	Asn	Ser	Ile	Ile	Asp	Lys		
				385					390					395			400

Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu Glu		
405	410	415
Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp		
420	425	430
Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg		
435	440	445
Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val		
450	455	460
Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe		
465	470	475
Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Ile Arg Asn		
485	490	495
Gly Thr Tyr Asn Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Lys Arg		
500	505	510
Glu Glu Ile Ser Ser Gly Gly Glu Ser Gln Val Arg Gln Gln Phe Ser		
515	520	525
Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln		
530	535	540
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser		
545	550	555
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr		
565	570	575
Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro		
580	585	590
Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu		
595	600	605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu		
610	615	620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp His Ala		
625	630	635
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu		
645	650	655
Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu		
660	665	670
Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys		
675	680	685
Ser Arg Lys Ser		
690		

<210> 21

<211> 693

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 21

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Met Lys Ala Lys Leu Leu Ile Leu Leu Cys Ala Leu Ser Ala Thr Asp
1           5           10           15
Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
          20           25           30
Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
          35           40           45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Arg Leu Lys Gly Lys
          50           55           60
Ala Pro Leu Gln Leu Gly Asn Cys Asn Ile Ala Gly Trp Val Leu Gly
65           70           75           80
Asn Pro Glu Cys Glu Ser Leu Leu Ser Asn Arg Ser Trp Ser Tyr Ile
          85           90           95
Ala Glu Thr Pro Asn Ser Glu Asn Gly Thr Cys Phe Pro Gly Asp Phe
          100          105          110
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
          115          120          125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Arg Ser Trp Pro Asn His Thr
          130          135          140
Thr Arg Gly Val Thr Ala Ala Cys Pro His Ala Arg Lys Ser Ser Phe
145          150          155          160
Tyr Lys Asn Leu Val Trp Leu Thr Glu Ala Asn Gly Ser Tyr Pro Asn
          165          170          175
Leu Ser Arg Ser Tyr Val Asn Asn Gln Glu Lys Glu Val Leu Val Leu
          180          185          190
Trp Gly Val His His Pro Ser Asn Ile Glu Glu Gln Arg Ala Leu Tyr
          195          200          205
Arg Lys Asp Asn Ala Tyr Val Ser Val Val Ser Ser Asn Tyr Asn Arg
          210          215          220
Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln Ser
225          230          235          240
Gly Arg Met Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr Ile

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	245		250		255
Ile Phe Glu Ala Thr Gly Asn Leu	Ile Ala Pro Trp Tyr Ala Phe Ala				
260	265	270			
Leu Ser Arg Gly Pro Gly Ser Gly	Ile Ile Thr Ser Asn Ala Pro Leu				
275	280	285			
Asp Glu Cys Asp Thr Lys Cys Gln Thr	Pro Gln Gly Ala Ile Asn Ser				
290	295	300			
Ser Leu Pro Phe Gln Asn Ile His Pro	Val Thr Ile Gly Glu Cys Pro				
305	310	315			320
Lys Tyr Val Arg Ser Thr Lys Leu Arg	Met Val Thr Gly Leu Arg Asn				
325	330	335			
Ile Pro Ser Val Gln Ser Arg Gly Leu	Phe Gly Ala Ile Ala Gly Phe				
340	345	350			
Ile Glu Gly Gly Trp Thr Gly Met Met	Asp Gly Trp Tyr Gly Tyr His				
355	360	365			
His Gln Asn Glu Gln Gly Ser Gly Tyr	Ala Ala Asp Gln Lys Ser Thr				
370	375	380			
Gln Asn Ala Ile Asn Gly Ile Thr Asn	Lys Val Asn Ser Val Ile Glu				
385	390	395			400
Lys Met Asn Thr Gln Phe Thr Ala Val	Gly Lys Glu Phe Asn Lys Leu				
405	410	415			
Glu Lys Arg Met Glu Asn Leu Asn Lys	Lys Val Asp Asp Gly Phe Met				
420	425	430			
Asp Ile Trp Thr Tyr Asn Ala Glu Leu	Leu Val Leu Leu Glu Asn Glu				
435	440	445			
Arg Thr Leu Asp Phe His Asp Ser Asn	Val Lys Asn Leu Tyr Glu Lys				
450	455	460			
Val Lys Asn Gln Leu Arg Asn Asn Ala	Lys Glu Leu Gly Asn Gly Cys				
465	470	475			480
Phe Glu Phe Tyr His Lys Cys Asp Asn	Glu Cys Met Glu Ser Val Lys				
485	490	495			
Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr	Ser Glu Glu Ser Lys Leu Asn				
500	505	510			
Arg Glu Lys Ile Asp Ser Gly Gly Glu	Ser Gln Val Arg Gln Gln Phe				
515	520	525			
Ser Lys Asp Ile Glu Lys Leu Leu Asn	Glu Gln Val Asn Lys Glu Met				
530	535	540			
Gln Ser Ser Asn Leu Tyr Met Ser Met	Ser Ser Trp Ser Tyr Thr His				
545	550	555			560

Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu
 565 570 575
 Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val
 580 585 590
 Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly
 595 600 605
 Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser
 610 615 620
 Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp His
 625 630 635 640
 Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu
 645 650 655
 Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn
 660 665 670
 Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala
 675 680 685
 Lys Ser Arg Lys Ser
 690

<210> 22

<211> 694

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 22

Met Lys Thr Thr Ile Ile Leu Ile Leu Leu Thr His Trp Ala Tyr Ser
 1 5 10 15
 Gln Asn Pro Ile Ser Gly Asn Asn Thr Ala Thr Leu Cys Leu Gly His
 20 25 30
 His Ala Val Ala Asn Gly Thr Leu Val Lys Thr Ile Ser Asp Asp Gln
 35 40 45
 Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ile Ser Met Gly
 50 55 60
 Lys Ile Cys Asn Asn Ser Tyr Arg Ile Leu Asp Gly Arg Asn Cys Thr
 65 70 75 80
 Leu Ile Asp Ala Met Leu Gly Asp Pro His Cys Asp Ala Phe Gln Tyr
 85 90 95

Glu Asn Trp Asp Leu Phe Ile Glu Arg Ser Ser Ala Phe Ser Asn Cys		
100	105	110
Phe Pro Tyr Asp Ile Pro Asp Tyr Ala Ser Leu Arg Ser Ile Val Ala		
115	120	125
Ser Ser Gly Thr Leu Glu Phe Thr Ala Glu Gly Phe Thr Trp Thr Gly		
130	135	140
Val Thr Gln Asn Gly Arg Ser Gly Ala Cys Lys Arg Gly Ser Ala Asp		
145	150	155
Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr Lys Ser Gly Ser Ser Tyr		
165	170	175
Pro Thr Leu Asn Val Thr Met Pro Asn Asn Lys Asn Phe Asp Lys Leu		
180	185	190
Tyr Ile Trp Gly Ile His His Pro Ser Ser Asn Gln Glu Gln Thr Lys		
195	200	205
Leu Tyr Ile Gln Glu Ser Gly Arg Val Thr Val Ser Thr Lys Arg Ser		
210	215	220
Gln Gln Thr Ile Ile Pro Asn Ile Gly Ser Arg Pro Trp Val Arg Gly		
225	230	235
Gln Ser Gly Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly Asp		
245	250	255
Ile Leu Met Ile Asn Ser Asn Gly Asn Leu Val Ala Pro Arg Gly Tyr		
260	265	270
Phe Lys Leu Lys Thr Gly Lys Ser Ser Val Met Arg Ser Asp Val Pro		
275	280	285
Ile Asp Ile Cys Val Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile Ser		
290	295	300
Asn Asp Lys Pro Phe Gln Asn Val Asn Lys Val Thr Tyr Gly Lys Cys		
305	310	315
Pro Lys Tyr Ile Arg Gln Asn Thr Leu Lys Leu Ala Thr Gly Met Arg		
325	330	335
Asn Val Pro Glu Lys Gln Ile Arg Gly Ile Phe Gly Ala Ile Ala Gly		
340	345	350
Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly Phe		
355	360	365
Arg Tyr Gln Asn Ser Glu Gly Thr Gly Gln Ala Ala Asp Leu Lys Ser		
370	375	380
Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Val Ile		
385	390	395
Glu Arg Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser Glu		

	405	410	415
Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr Lys			
	420	425	430
Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu Asn			
	435	440	445
Gln His Thr Ile Asp Leu Thr Asp Ala Glu Met Asn Lys Leu Phe Glu			
	450	455	460
Lys Thr Arg Arg Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Gly Gly			
465	470	475	480
Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Gly Ser Ile			
	485	490	495
Arg Asn Gly Thr Tyr Asp His Tyr Ile Tyr Arg Asp Glu Ala Leu Asn			
	500	505	510
Asn Arg Phe Lys Ile Asp Ser Gly Gly Glu Ser Gln Val Arg Gln Gln			
	515	520	525
Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu			
	530	535	540
Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr			
545	550	555	560
His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu			
	565	570	575
Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn			
	580	585	590
Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu			
	595	600	605
Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile			
	610	615	620
Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp			
625	630	635	640
His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu			
	645	650	655
Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly			
	660	665	670
Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile			
	675	680	685
Ala Lys Ser Arg Lys Ser			
	690		
<210> 23			
<211> 696			

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 23

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Met Lys Thr Thr Ile Ile Phe Ile Phe Ile Leu Leu Thr His Trp Ala
1           5           10           15
Tyr Ser Gln Asn Pro Ile Ser Asp Asn Asn Thr Ala Thr Leu Cys Leu
          20           25           30
Gly His His Ala Val Ala Asn Gly Thr Leu Val Lys Thr Ile Ser Asp
          35           40           45
Asp Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ile Ser
          50           55           60
Met Gly Lys Ile Cys Asn Asn Ser Tyr Arg Ile Leu Asp Gly Arg Asn
65           70           75           80
Cys Thr Leu Ile Asp Ala Met Leu Gly Asp Pro His Cys Asp Val Phe
          85           90           95
Gln Tyr Glu Asn Trp Asp Leu Phe Ile Glu Arg Ser Ser Ala Phe Ser
          100          105          110
Asn Cys Phe Pro Tyr Asp Ile Pro Asp Tyr Ala Ser Leu Arg Ser Ile
          115          120          125
Val Ala Ser Ser Gly Thr Leu Glu Phe Thr Ala Glu Gly Phe Thr Trp
          130          135          140
Thr Gly Val Thr Gln Asn Gly Arg Ser Gly Ala Cys Lys Arg Gly Ser
145          150          155          160
Ala Asp Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr Lys Ser Gly Asn
          165          170          175
Ser Tyr Pro Thr Leu Asn Val Thr Met Pro Asn Asn Lys Asn Phe Asp
          180          185          190
Lys Leu Tyr Ile Trp Gly Ile His His Pro Ser Ser Asn Gln Glu Gln
          195          200          205
Thr Lys Leu Tyr Ile Gln Glu Ser Gly Arg Val Thr Val Ser Thr Lys
          210          215          220
Arg Ser Gln Gln Thr Met Ile Pro Asn Ile Gly Ser Arg Pro Trp Val
225          230          235          240
Arg Gly Gln Ser Gly Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro
          245          250          255

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Gly Asp Ile Leu Met Ile Asn Ser Asn Gly Asn Leu Val Ala Pro Arg		
260	265	270
Gly Tyr Phe Lys Leu Lys Thr Gly Lys Ser Ser Val Met Arg Ser Asp		
275	280	285
Val Pro Ile Asp Ile Cys Val Ser Glu Cys Ile Thr Pro Asn Gly Ser		
290	295	300
Ile Ser Asn Asp Lys Pro Phe Gln Asn Val Asn Lys Val Thr Tyr Gly		
305	310	315
Lys Cys Pro Lys Tyr Ile Arg Gln Asn Thr Leu Lys Leu Ala Thr Gly		
325	330	335
Met Arg Asn Val Pro Glu Lys Gln Ile Arg Gly Ile Phe Gly Ala Ile		
340	345	350
Ala Gly Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr		
355	360	365
Gly Phe Arg Tyr Gln Asn Ser Glu Gly Thr Gly Gln Ala Ala Asp Leu		
370	375	380
Lys Ser Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg		
385	390	395
Val Ile Glu Arg Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe		
405	410	415
Ser Glu Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp		
420	425	430
Thr Lys Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu		
435	440	445
Glu Asn Gln His Thr Ile Asp Leu Thr Asp Ala Glu Met Asn Lys Leu		
450	455	460
Phe Glu Lys Thr Arg Arg Gln Leu Arg Glu Asn Ala Glu Asp Met Gly		
465	470	475
Gly Gly Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Gly		
485	490	495
Ser Ile Arg Asn Gly Thr Tyr Asp His Tyr Ile Tyr Arg Asp Glu Ala		
500	505	510
Leu Asn Asn Arg Phe Lys Ile Asp Ser Gly Gly Glu Ser Gln Val Arg		
515	520	525
Gln Gln Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn		
530	535	540
Lys Glu Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser		
545	550	555
Tyr Thr His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala		

565	570	575
Ala Glu Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu		
580	585	590
Asn Asn Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys		
595	600	605
Phe Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln		
610	615	620
His Ile Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys		
625	630	635
Lys Asp His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln		
645	650	655
His Glu Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu		
660	665	670
Ile Gly Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys		
675	680	685
Gly Ile Ala Lys Ser Arg Lys Ser		
690	695	
<210> 24		
<211> 694		
<212> PRT		
<213> 人工序列		
<220>		
<221> 来源		
<223> /注释="人工序列的描述：合成多肽"		
<400> 24		
Met Glu Thr Thr Ile Ile Leu Ile Leu Leu Thr His Trp Val Tyr Ser		
1 5 10 15		
Gln Asn Pro Ile Ser Gly Asn Asn Thr Ala Thr Leu Cys Leu Gly His		
20 25 30		
His Ala Val Ala Asn Gly Thr Leu Val Lys Thr Ile Thr Asp Asp Gln		
35 40 45		
Ile Glu Val Thr Asn Ala Thr Glu Leu Val Glu Ser Ile Ser Met Gly		
50 55 60		
Lys Ile Cys Asn Asn Ser Tyr Arg Val Leu Asp Gly Arg Asn Cys Thr		
65 70 75 80		
Leu Ile Asp Ala Met Leu Gly Asp Pro His Cys Asp Asp Phe Gln Tyr		
85 90 95		
Glu Ser Trp Asp Leu Phe Ile Glu Arg Ser Ser Ala Ser Ser Asn Cys		

100	105	110
Phe Pro Tyr Asp Ile Pro Asp Tyr Ala Ser Leu Arg Ser Ile Val Ala		
115	120	125
Ser Ser Gly Thr Leu Glu Phe Thr Ala Glu Gly Phe Thr Trp Thr Gly		
130	135	140
Val Thr Gln Asn Gly Arg Ser Gly Ala Cys Lys Arg Gly Ser Ala Asp		
145	150	155
Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr Lys Ser Gly Asn Ser Tyr		
165	170	175
Pro Thr Leu Asn Val Thr Met Pro Asn Asn Lys Asn Phe Asp Lys Leu		
180	185	190
Tyr Ile Trp Gly Ile His His Pro Ser Ser Asn Lys Glu Gln Thr Lys		
195	200	205
Leu Tyr Ile Gln Glu Ser Gly Arg Val Thr Val Ser Thr Glu Arg Ser		
210	215	220
Gln Gln Thr Val Ile Pro Asn Ile Gly Ser Arg Pro Trp Val Arg Gly		
225	230	235
Gln Ser Gly Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly Asp		
245	250	255
Val Leu Met Ile Asn Ser Asn Gly Asn Leu Val Ala Pro Arg Gly Tyr		
260	265	270
Phe Lys Leu Arg Thr Gly Lys Ser Ser Val Met Arg Ser Asp Ala Leu		
275	280	285
Ile Asp Thr Cys Val Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile Pro		
290	295	300
Asn Asp Lys Pro Phe Gln Asn Val Asn Lys Ile Thr Tyr Gly Arg Cys		
305	310	315
Pro Lys Tyr Ile Arg Gln Asn Thr Leu Lys Leu Ala Thr Gly Met Arg		
325	330	335
Asn Val Pro Glu Lys Gln Ile Arg Gly Ile Phe Gly Ala Ile Ala Gly		
340	345	350
Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly Phe		
355	360	365
Arg Tyr Gln Asn Ser Glu Gly Thr Gly Gln Ala Ala Asp Leu Lys Ser		
370	375	380
Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Val Ile		
385	390	395
Glu Arg Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser Glu		
405	410	415

Val	Glu	Gly	Arg	Ile	Gln	Asp	Leu	Glu	Lys	Tyr	Val	Glu	Asp	Thr	Lys
420				425				430							
Ile	Asp	Leu	Trp	Ser	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Ala	Leu	Glu	Asn
435				440				445							
Gln	His	Thr	Ile	Asp	Leu	Thr	Asp	Ala	Glu	Met	Asn	Lys	Leu	Phe	Glu
450				455				460							
Arg	Thr	Arg	Arg	Gln	Leu	Arg	Glu	Asn	Ala	Glu	Asp	Met	Gly	Gly	Gly
465				470				475				480			
Cys	Phe	Lys	Ile	Tyr	His	Lys	Cys	Asp	Asn	Ala	Cys	Ile	Gly	Ser	Ile
485				490				495							
Arg	Asn	Gly	Thr	Tyr	Asp	His	Tyr	Ile	Tyr	Arg	Asp	Glu	Ala	Leu	Asn
500				505				510							
Asn	Arg	Phe	Lys	Ile	Asp	Ser	Gly	Gly	Glu	Ser	Gln	Val	Arg	Gln	Gln
515				520				525							
Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu	Asn	Glu	Gln	Val	Asn	Lys	Glu
530				535				540							
Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met	Ser	Ser	Trp	Ser	Tyr	Thr
545				550				555				560			
His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu	Phe	Asp	His	Ala	Ala	Glu
565				570				575							
Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe	Leu	Asn	Glu	Asn	Asn
580				585				590							
Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	Ala	Pro	Glu	His	Lys	Phe	Glu
595				600				605							
Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr	Glu	His	Glu	Gln	His	Ile
610				615				620							
Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	His	Ala	Ile	Lys	Cys	Lys	Asp
625				630				635				640			
His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr	Val	Ala	Glu	Gln	His	Glu
645				650				655							
Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp	Lys	Ile	Glu	Leu	Ile	Gly
660				665				670							
Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	Asp	Gln	Tyr	Val	Lys	Gly	Ile
675				680				685							
Ala	Lys	Ser	Arg	Lys	Ser										
690															
<210> 25															
<211> 711															
<212> PRT															

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 25

Met	Lys	Ala	Ile	Ile	Val	Leu	Leu	Met	Val	Val	Thr	Ser	Asn	Ala	Asp
1				5					10					15	
Arg	Ile	Cys	Thr	Gly	Ile	Thr	Ser	Ser	Asn	Ser	Pro	His	Val	Val	Lys
			20					25					30		
Thr	Ala	Thr	Gln	Gly	Glu	Val	Asn	Val	Thr	Gly	Val	Ile	Pro	Leu	Thr
			35				40					45			
Thr	Thr	Pro	Thr	Lys	Ser	Tyr	Phe	Ala	Asn	Leu	Lys	Gly	Thr	Lys	Thr
	50					55				60					
Arg	Gly	Lys	Leu	Cys	Pro	Asp	Cys	Leu	Asn	Cys	Thr	Asp	Leu	Asp	Val
65				70					75					80	
Ala	Leu	Gly	Arg	Pro	Met	Cys	Val	Gly	Thr	Thr	Pro	Ser	Ala	Lys	Ala
				85				90					95		
Ser	Ile	Leu	His	Glu	Val	Arg	Pro	Val	Thr	Ser	Gly	Cys	Phe	Pro	Ile
			100					105					110		
Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Ala	Asn	Leu	Leu	Arg	Gly
	115					120					125				
Tyr	Glu	Asn	Ile	Arg	Leu	Ser	Thr	Gln	Asn	Val	Ile	Asp	Ala	Glu	Lys
	130					135				140					
Ala	Pro	Gly	Gly	Pro	Tyr	Arg	Leu	Gly	Thr	Ser	Gly	Ser	Cys	Pro	Asn
145				150					155					160	
Ala	Thr	Ser	Lys	Ser	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	Pro
			165					170					175		
Lys	Asp	Asn	Asn	Lys	Asn	Ala	Thr	Asn	Pro	Leu	Thr	Val	Glu	Val	Pro
			180					185					190		
Tyr	Ile	Cys	Ala	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe	His
	195					200					205				
Ser	Asp	Asn	Lys	Thr	Gln	Met	Lys	Asn	Leu	Tyr	Gly	Asp	Ser	Asn	Pro
	210					215				220					
Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val	Ser
225				230					235					240	
Gln	Ile	Gly	Gly	Phe	Pro	Asp	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro	Gln
			245					250					255		
Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Met	Gln	Lys	Pro	Gly	Lys	Thr

260	265	270
Gly Thr Ile Val Tyr Gln Arg	Gly Val Leu Leu Pro Gln Lys Val Trp	
275	280	285
Cys Ala Ser Gly Arg Ser Lys	Val Ile Lys Gly Ser Leu Pro Leu Ile	
290	295	300
Gly Glu Ala Asp Cys Leu His	Glu Lys Tyr Gly Gly Leu Asn Lys Ser	
305	310	315
Lys Pro Tyr Tyr Thr Gly Glu His	Ala Lys Ala Ile Gly Asn Cys Pro	
325	330	335
Ile Trp Val Lys Thr Pro Leu Lys	Leu Ala Asn Gly Thr Lys Tyr Arg	
340	345	350
Pro Pro Ala Lys Leu Leu Lys	Glu Arg Gly Phe Phe Gly Ala Ile Ala	
355	360	365
Gly Phe Leu Glu Gly Gly Trp	Glu Gly Met Ile Ala Gly Trp His Gly	
370	375	380
Tyr Thr Ser His Gly Ala His	Gly Val Ala Val Ala Ala Asp Leu Lys	
385	390	395
Ser Thr Gln Glu Ala Ile Asn	Lys Ile Thr Lys Asn Leu Asn Ser Leu	
405	410	415
Ser Glu Leu Glu Val Lys Asn	Leu Gln Arg Leu Ser Gly Ala Met Asp	
420	425	430
Glu Leu His Asn Glu Ile Leu	Glu Leu Asp Glu Lys Val Asp Asp Leu	
435	440	445
Arg Ala Asp Thr Ile Ser Ser	Gln Ile Glu Leu Ala Val Leu Leu Ser	
450	455	460
Asn Glu Gly Ile Ile Asn Ser	Glu Asp Glu His Leu Leu Ala Leu Glu	
465	470	475
Arg Lys Leu Lys Lys Met Leu	Gly Pro Ser Ala Val Asp Ile Gly Asn	
485	490	495
Gly Cys Phe Glu Thr Lys His	Lys Cys Asn Gln Thr Cys Leu Asp Arg	
500	505	510
Ile Ala Ala Gly Thr Phe Asn	Ala Gly Glu Phe Ser Leu Pro Thr Phe	
515	520	525
Asp Ser Leu Asn Ile Thr Ala	Ser Gly Gly Glu Ser Gln Val Arg Gln	
530	535	540
Gln Phe Ser Lys Asp Ile Glu	Lys Leu Leu Asn Glu Gln Val Asn Lys	
545	550	555
Glu Met Gln Ser Ser Asn Leu	Tyr Met Ser Met Ser Ser Trp Ser Tyr	
565	570	575

Thr His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala		
580	585	590
Glu Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn		
595	600	605
Asn Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe		
610	615	620
Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His		
625	630	635
Ile Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys		
645	650	655
Asp His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His		
660	665	670
Glu Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile		
675	680	685
Gly Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly		
690	695	700
Ile Ala Lys Ser Arg Lys Ser		
705	710	

<210> 26

<211> 713

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 26

Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp		
1	5	10
Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys		
20	25	30
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr		
35	40	45
Thr Thr Pro Thr Lys Ser Tyr Phe Ala Asn Leu Lys Gly Thr Arg Thr		
50	55	60
Arg Gly Lys Leu Cys Pro Asp Cys Leu Asn Cys Thr Asp Leu Asp Val		
65	70	75
Ala Leu Gly Arg Pro Met Cys Val Gly Thr Thr Pro Ser Ala Lys Ala		
85	90	95

Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile		
100	105	110
Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly		
115	120	125
Tyr Glu Lys Ile Arg Leu Ser Thr Gln Asn Val Ile Asp Ala Glu Lys		
130	135	140
Ala Pro Gly Gly Pro Tyr Arg Leu Gly Thr Ser Gly Ser Cys Pro Asn		
145	150	155
Ala Thr Ser Lys Ile Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro		
165	170	175
Lys Asp Asn Tyr Lys Asn Ala Thr Asn Pro Leu Thr Val Glu Val Pro		
180	185	190
Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly Phe His		
195	200	205
Ser Asp Asn Lys Thr Gln Met Lys Ser Leu Tyr Gly Asp Ser Asn Pro		
210	215	220
Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr Val Ser		
225	230	235
Gln Ile Gly Asp Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro Gln		
245	250	255
Ser Gly Arg Ile Val Val Asp Tyr Met Met Gln Lys Pro Gly Lys Thr		
260	265	270
Gly Thr Ile Val Tyr Gln Arg Gly Val Leu Leu Pro Gln Lys Val Trp		
275	280	285
Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile		
290	295	300
Gly Glu Ala Asp Cys Leu His Glu Glu Tyr Gly Gly Leu Asn Lys Ser		
305	310	315
Lys Pro Tyr Tyr Thr Gly Lys His Ala Lys Ala Ile Gly Asn Cys Pro		
325	330	335
Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg		
340	345	350
Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile Ala		
355	360	365
Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly		
370	375	380
Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys		
385	390	395
Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu		

			405				410				415				
Ser	Glu	Leu	Glu	Val	Lys	Asn	Leu	Gln	Arg	Leu	Ser	Gly	Ala	Met	Asp
			420				425				430				
Glu	Leu	His	Asn	Glu	Ile	Leu	Glu	Leu	Asp	Glu	Lys	Val	Asp	Asp	Leu
			435				440				445				
Arg	Ala	Asp	Thr	Ile	Ser	Ser	Gln	Ile	Glu	Leu	Ala	Val	Leu	Leu	Ser
			450				455				460				
Asn	Glu	Gly	Ile	Ile	Asn	Ser	Glu	Asp	Glu	His	Leu	Leu	Ala	Leu	Glu
465			470				475				480				
Arg	Lys	Leu	Lys	Lys	Met	Leu	Gly	Pro	Ser	Ala	Val	Asp	Ile	Gly	Asn
			485				490				495				
Gly	Cys	Phe	Glu	Thr	Lys	His	Lys	Cys	Asn	Gln	Thr	Cys	Leu	Asp	Arg
			500				505				510				
Ile	Ala	Ala	Gly	Thr	Phe	Asn	Ala	Gly	Glu	Phe	Ser	Leu	Pro	Thr	Phe
			515				520				525				
Asp	Ser	Leu	Asn	Ile	Thr	Ala	Ala	Ser	Ser	Gly	Gly	Glu	Ser	Gln	Val
530			535				540								
Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu	Asn	Glu	Gln	Val
545			550				555				560				
Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met	Ser	Ser	Trp
			565				570				575				
Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu	Phe	Asp	His
			580				585				590				
Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe	Leu	Asn
			595				600				605				
Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	Ala	Pro	Glu	His
610			615				620								
Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr	Glu	His	Glu
625			630				635				640				
Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	His	Ala	Ile	Lys
			645				650				655				
Cys	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr	Val	Ala	Glu
			660				665				670				
Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp	Lys	Ile	Glu
			675				680				685				
Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	Asp	Gln	Tyr	Val
690			695				700								
Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser							
705			710												

<210> 27

<211> 694

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 27

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Met Lys Ala Arg Leu Leu Val Leu Leu Cys Ala Leu Ala Ala Thr Asp
1           5           10           15
Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
          20           25           30
Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
          35           40           45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Lys Leu Lys Gly Ile
          50           55           60
Ala Pro Leu Gln Leu Gly Lys Cys Asn Ile Ala Gly Trp Leu Leu Gly
65           70           75           80
Asn Pro Glu Cys Glu Ser Leu Leu Ser Ala Arg Ser Trp Ser Tyr Ile
          85           90           95
Val Glu Thr Pro Asn Ser Glu Asn Gly Thr Cys Phe Pro Gly Asp Phe
          100          105          110
Ile Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe
          115          120          125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Asn
          130          135          140
Thr Thr Lys Gly Val Thr Ala Ala Cys Ser His Ala Gly Lys Ser Ser
145          150          155          160
Phe Tyr Arg Asn Leu Leu Trp Leu Thr Lys Lys Gly Gly Ser Tyr Pro
          165          170          175
Lys Leu Ser Lys Ser Tyr Val Asn Asn Lys Gly Lys Glu Val Leu Val
          180          185          190
Leu Trp Gly Val His His Pro Ser Thr Ser Thr Asp Gln Gln Ser Leu
          195          200          205
Tyr Gln Asn Glu Asn Ala Tyr Val Ser Val Val Ser Ser Asn Tyr Asn
          210          215          220
Arg Arg Phe Thr Pro Glu Ile Ala Glu Arg Pro Lys Val Arg Gly Gln
225          230          235          240

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Ala Gly Arg Met Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr		
245	250	255
Ile Ile Phe Glu Ala Thr Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe		
260	265	270
Ala Leu Ser Arg Gly Ser Gly Ser Gly Ile Ile Thr Ser Asn Ala Ser		
275	280	285
Met His Glu Cys Asn Thr Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn		
290	295	300
Ser Ser Leu Pro Phe Gln Asn Ile His Pro Val Thr Ile Gly Glu Cys		
305	310	315
Pro Lys Tyr Val Arg Ser Thr Lys Leu Arg Met Val Thr Gly Leu Arg		
325	330	335
Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly		
340	345	350
Phe Ile Glu Gly Gly Trp Thr Gly Met Ile Asp Gly Trp Tyr Gly Tyr		
355	360	365
His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser		
370	375	380
Thr Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile		
385	390	395
Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Asn		
405	410	415
Leu Glu Lys Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe		
420	425	430
Leu Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn		
435	440	445
Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu		
450	455	460
Lys Val Lys Ser Gln Leu Arg Asn Asn Ala Lys Glu Ile Gly Asn Gly		
465	470	475
Cys Phe Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Val		
485	490	495
Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu		
500	505	510
Asn Arg Glu Lys Ile Asp Ser Gly Gly Glu Ser Gln Val Arg Gln Gln		
515	520	525
Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu		
530	535	540
Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr		

545	550	555	560
His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu			
	565	570	575
Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn			
	580	585	590
Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu			
	595	600	605
Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile			
610	615	620	
Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp			
625	630	635	640
His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu			
	645	650	655
Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly			
	660	665	670
Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile			
	675	680	685
Ala Lys Ser Arg Lys Ser			
690			
<210> 28			
<211> 694			
<212> PRT			
<213> 人工序列			
<220>			
<221> 来源			
<223> /注释="人工序列的描述：合成多肽"			
<400> 28			
Met Glu Ala Arg Leu Leu Val Leu Leu Cys Ala Phe Ala Ala Thr Asn			
1	5	10	15
Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr			
	20	25	30
Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn			
	35	40	45
Leu Leu Glu Asp Ser His Asn Gly Lys Leu Cys Arg Leu Lys Gly Ile			
	50	55	60
Ala Pro Leu Gln Leu Gly Asn Cys Ser Val Ala Gly Trp Ile Leu Gly			
65	70	75	80
Asn Pro Glu Cys Glu Ser Leu Phe Ser Lys Glu Ser Trp Ser Tyr Ile			

85	90	95
Ala Glu Thr Pro Asn Pro Glu Asn Gly Thr Cys Phe Pro Gly Tyr Phe		
100	105	110
Ala Asp Tyr Glu Glu Leu Arg Glu Gln Leu Ser Ser Val Ser Ser Phe		
115	120	125
Glu Arg Phe Glu Ile Phe Pro Lys Glu Ser Ser Trp Pro Asn His Thr		
130	135	140
Val Thr Lys Gly Val Thr Ala Ser Cys Ser His Asn Gly Lys Ser Ser		
145	150	155
Phe Tyr Arg Asn Leu Leu Trp Leu Thr Glu Lys Asn Gly Leu Tyr Pro		
165	170	175
Asn Leu Ser Lys Ser Tyr Val Asn Asn Lys Glu Lys Glu Val Leu Val		
180	185	190
Leu Trp Gly Val His His Pro Ser Asn Ile Gly Asp Gln Arg Ala Ile		
195	200	205
Tyr His Thr Glu Asn Ala Tyr Val Ser Val Val Ser Ser His Tyr Ser		
210	215	220
Arg Arg Phe Thr Pro Glu Ile Ala Lys Arg Pro Lys Val Arg Asp Gln		
225	230	235
Glu Gly Arg Ile Asn Tyr Tyr Trp Thr Leu Leu Glu Pro Gly Asp Thr		
245	250	255
Ile Ile Phe Glu Ala Asn Gly Asn Leu Ile Ala Pro Trp Tyr Ala Phe		
260	265	270
Ala Leu Ser Arg Gly Phe Gly Ser Gly Ile Ile Thr Ser Asn Ala Ser		
275	280	285
Met Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn		
290	295	300
Ser Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys		
305	310	315
Pro Lys Tyr Val Arg Ser Thr Lys Leu Arg Met Val Thr Gly Leu Arg		
325	330	335
Asn Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly		
340	345	350
Phe Ile Glu Gly Gly Trp Thr Gly Met Ile Asp Gly Trp Tyr Gly Tyr		
355	360	365
His His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser		
370	375	380
Thr Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile		
385	390	395
		400

Glu Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys		
405	410	415
Leu Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe		
420	425	430
Leu Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn		
435	440	445
Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu		
450	455	460
Lys Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly		
465	470	475
Cys Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val		
485	490	495
Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu		
500	505	510
Asn Arg Glu Lys Ile Asp Ser Gly Gly Glu Ser Gln Val Arg Gln Gln		
515	520	525
Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu		
530	535	540
Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr		
545	550	555
His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu		
565	570	575
Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn		
580	585	590
Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu		
595	600	605
Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile		
610	615	620
Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp		
625	630	635
His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu		
645	650	655
Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly		
660	665	670
Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile		
675	680	685
Ala Lys Ser Arg Lys Ser		
690		

<210> 29

<211> 693

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 29

Met	Glu	Ala	Arg	Leu	Leu	Val	Leu	Leu	Cys	Ala	Phe	Ala	Ala	Thr	Asn
1				5					10					15	
Ala	Asp	Thr	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Asp	Thr
			20					25					30		
Val	Asp	Thr	Val	Leu	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ser	Val	Asn
			35				40					45			
Leu	Leu	Glu	Asp	Ser	His	Asn	Gly	Lys	Leu	Cys	Leu	Leu	Lys	Gly	Ile
			50				55				60				
Ala	Pro	Leu	Gln	Leu	Gly	Asn	Cys	Ser	Val	Ala	Gly	Trp	Ile	Leu	Gly
65				70					75					80	
Asn	Pro	Glu	Cys	Glu	Leu	Leu	Ile	Ser	Lys	Glu	Ser	Trp	Ser	Tyr	Ile
			85					90					95		
Val	Glu	Thr	Pro	Asn	Pro	Glu	Asn	Gly	Thr	Cys	Phe	Pro	Gly	Tyr	Phe
			100					105					110		
Ala	Asp	Tyr	Glu	Glu	Leu	Arg	Glu	Gln	Leu	Ser	Ser	Val	Ser	Ser	Phe
			115					120					125		
Glu	Arg	Phe	Glu	Ile	Phe	Pro	Lys	Glu	Ser	Ser	Trp	Pro	Asn	His	Thr
			130				135					140			
Val	Thr	Gly	Val	Ser	Ala	Ser	Cys	Ser	His	Asn	Gly	Lys	Ser	Ser	Phe
145				150					155					160	
Tyr	Arg	Asn	Leu	Leu	Trp	Leu	Thr	Gly	Lys	Asn	Gly	Leu	Tyr	Pro	Asn
			165					170					175		
Leu	Ser	Lys	Ser	Tyr	Ala	Asn	Asn	Lys	Glu	Lys	Glu	Val	Leu	Val	Leu
			180					185					190		
Trp	Gly	Val	His	His	Pro	Pro	Asn	Ile	Gly	Asp	Gln	Arg	Ala	Leu	Tyr
			195				200					205			
His	Thr	Glu	Asn	Ala	Tyr	Val	Ser	Val	Val	Ser	Ser	His	Tyr	Ser	Arg
			210				215					220			
Lys	Phe	Thr	Pro	Glu	Ile	Ala	Lys	Arg	Pro	Lys	Val	Arg	Asp	Gln	Glu
225				230					235					240	
Gly	Arg	Ile	Asn	Tyr	Tyr	Trp	Thr	Leu	Leu	Glu	Pro	Gly	Asp	Thr	Ile

	245		250		255
Ile Phe Glu Ala Asn Gly Asn Leu	Ile Ala Pro Arg Tyr Ala Phe Ala				
260	265	270			
Leu Ser Arg Gly Phe Gly Ser Gly	Ile Ile Thr Ser Asn Ala Pro Met				
275	280	285			
Asp Glu Cys Asp Ala Lys Cys Gln Thr Pro Gln Gly Ala Ile Asn Ser					
290	295	300			
Ser Leu Pro Phe Gln Asn Val His Pro Val Thr Ile Gly Glu Cys Pro					
305	310	315			320
Lys Tyr Val Arg Ser Ala Lys Leu Arg Met Val Thr Gly Leu Arg Asn					
325	330	335			
Ile Pro Ser Ile Gln Ser Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe					
340	345	350			
Ile Glu Gly Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His					
355	360	365			
His Gln Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr					
370	375	380			
Gln Asn Ala Ile Asn Gly Ile Thr Asn Lys Val Asn Ser Val Ile Glu					
385	390	395			400
Lys Met Asn Thr Gln Phe Thr Ala Val Gly Lys Glu Phe Asn Lys Leu					
405	410	415			
Glu Arg Arg Met Glu Asn Leu Asn Lys Lys Val Asp Asp Gly Phe Leu					
420	425	430			
Asp Ile Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Leu Glu Asn Glu					
435	440	445			
Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Glu Lys					
450	455	460			
Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn Gly Cys					
465	470	475			480
Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser Val Lys					
485	490	495			
Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys Leu Asn					
500	505	510			
Arg Glu Lys Ile Asp Ser Gly Gly Glu Ser Gln Val Arg Gln Gln Phe					
515	520	525			
Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met					
530	535	540			
Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His					
545	550	555			560

Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu
 565 570 575
 Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val
 580 585 590
 Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly
 595 600 605
 Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser
 610 615 620
 Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp His
 625 630 635 640
 Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu
 645 650 655
 Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn
 660 665 670
 Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala
 675 680 685
 Lys Ser Arg Lys Ser
 690

<210> 30

<211> 695

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 30

Met Lys Thr Ile Ile Ala Leu Ser Tyr Ile Leu Cys Leu Val Phe Ala
 1 5 10 15
 Gln Lys Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
 20 25 30
 His His Ala Val Pro Asn Gly Thr Ile Val Lys Thr Ile Thr Asn Asp
 35 40 45
 Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Ser Ser Ser Thr
 50 55 60
 Gly Glu Ile Cys Asp Ser Pro His Gln Ile Leu Asp Gly Lys Asn Cys
 65 70 75 80
 Thr Leu Ile Asp Ala Leu Leu Gly Asp Pro Gln Cys Asp Gly Phe Gln
 85 90 95

Asn	Lys	Lys	Trp	Asp	Leu	Phe	Val	Glu	Arg	Ser	Lys	Ala	Tyr	Ser	Asn
100				105				110							
Cys	Tyr	Pro	Tyr	Asp	Val	Pro	Asp	Tyr	Ala	Ser	Leu	Arg	Ser	Leu	Val
115				120				125							
Ala	Ser	Ser	Gly	Thr	Leu	Glu	Phe	Asn	Asn	Glu	Ser	Phe	Asn	Trp	Thr
130				135				140							
Gly	Val	Thr	Gln	Asn	Gly	Thr	Ser	Ser	Ala	Cys	Ile	Arg	Arg	Ser	Lys
145				150				155				160			
Asn	Ser	Phe	Phe	Ser	Arg	Leu	Asn	Trp	Leu	Thr	His	Leu	Asn	Phe	Lys
165				170				175							
Tyr	Pro	Ala	Leu	Asn	Val	Thr	Met	Pro	Asn	Asn	Glu	Gln	Phe	Asp	Lys
180				185				190							
Leu	Tyr	Ile	Trp	Gly	Val	His	His	Pro	Gly	Thr	Asp	Lys	Asp	Gln	Ile
195				200				205							
Phe	Leu	Tyr	Ala	Gln	Ala	Ser	Gly	Arg	Ile	Thr	Val	Ser	Thr	Lys	Arg
210				215				220							
Ser	Gln	Gln	Thr	Val	Ser	Pro	Asn	Ile	Gly	Ser	Arg	Pro	Arg	Val	Arg
225				230				235				240			
Asn	Ile	Pro	Ser	Arg	Ile	Ser	Ile	Tyr	Trp	Thr	Ile	Val	Lys	Pro	Gly
245				250				255							
Asp	Ile	Leu	Leu	Ile	Asn	Ser	Thr	Gly	Asn	Leu	Ile	Ala	Pro	Arg	Gly
260				265				270							
Tyr	Phe	Lys	Ile	Arg	Ser	Gly	Lys	Ser	Ser	Ile	Met	Arg	Ser	Asp	Ala
275				280				285							
Pro	Ile	Gly	Lys	Cys	Asn	Ser	Glu	Cys	Ile	Thr	Pro	Asn	Gly	Ser	Ile
290				295				300							
Pro	Asn	Asp	Lys	Pro	Phe	Gln	Asn	Val	Asn	Arg	Ile	Thr	Tyr	Gly	Ala
305				310				315				320			
Cys	Pro	Arg	Tyr	Val	Lys	Gln	Asn	Thr	Leu	Lys	Leu	Ala	Thr	Gly	Met
325				330				335							
Arg	Asn	Val	Pro	Glu	Lys	Gln	Thr	Arg	Gly	Ile	Phe	Gly	Ala	Ile	Ala
340				345				350							
Gly	Phe	Ile	Glu	Asn	Gly	Trp	Glu	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly
355				360				365							
Phe	Arg	His	Gln	Asn	Ser	Glu	Gly	Arg	Gly	Gln	Ala	Ala	Asp	Leu	Lys
370				375				380							
Ser	Thr	Gln	Ala	Ala	Ile	Asp	Gln	Ile	Asn	Gly	Lys	Leu	Asn	Arg	Leu
385				390				395				400			
Ile	Gly	Lys	Thr	Asn	Glu	Lys	Phe	His	Gln	Ile	Glu	Lys	Glu	Phe	Ser

405	410	415
Glu Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr		
420	425	430
Lys Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu		
435	440	445
Asn Gln His Thr Ile Asp Leu Thr Asp Ser Glu Met Asn Lys Leu Phe		
450	455	460
Glu Lys Thr Lys Lys Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn		
465	470	475
Gly Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Gly Ser		
485	490	495
Ile Arg Asn Gly Thr Tyr Asp His Asp Val Tyr Arg Asp Glu Ala Leu		
500	505	510
Asn Asn Arg Phe Gln Ile Lys Ser Gly Gly Glu Ser Gln Val Arg Gln		
515	520	525
Gln Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys		
530	535	540
Glu Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr		
545	550	555
Thr His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala		
565	570	575
Glu Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn		
580	585	590
Asn Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe		
595	600	605
Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His		
610	615	620
Ile Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys		
625	630	635
Asp His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His		
645	650	655
Glu Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile		
660	665	670
Gly Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly		
675	680	685
Ile Ala Lys Ser Arg Lys Ser		
690	695	
<210> 31		
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<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 31

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Met Lys Thr Ile Ile Ala Leu Ser His Ile Leu Cys Leu Val Phe Ala
1           5           10           15
Gln Lys Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly
          20           25           30
His His Ala Val Pro Asn Gly Thr Ile Val Lys Thr Ile Thr Asn Asp
          35           40           45
Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Gln Asn Ser Ser Ile
          50           55           60
Gly Glu Ile Cys Asp Ser Pro His Gln Ile Leu Asp Gly Glu Asn Cys
65           70           75           80
Thr Leu Ile Asp Ala Leu Leu Gly Asp Pro Gln Cys Asp Gly Phe Gln
          85           90           95
Asn Lys Lys Trp Asp Leu Phe Val Glu Arg Ser Lys Ala Tyr Ser Asn
          100          105          110
Cys Tyr Pro Tyr Asp Val Pro Asp Tyr Ala Ser Leu Arg Ser Leu Val
          115          120          125
Ala Ser Ser Gly Thr Leu Glu Phe Asn Asn Glu Ser Phe Asn Trp Thr
          130          135          140
Gly Val Thr Gln Asn Gly Thr Ser Ser Ala Cys Ile Arg Arg Ser Asn
145          150          155          160
Asn Ser Phe Phe Ser Arg Leu Asn Trp Leu Thr Gln Leu Asn Phe Lys
          165          170          175
Tyr Pro Ala Leu Asn Val Thr Met Pro Asn Asn Glu Gln Phe Asp Lys
          180          185          190
Leu Tyr Ile Trp Gly Val His His Pro Val Thr Asp Lys Asp Gln Ile
          195          200          205
Phe Leu Tyr Ala Gln Ser Ser Gly Arg Ile Thr Val Ser Thr Lys Arg
          210          215          220
Ser Gln Gln Ala Val Ile Pro Asn Ile Gly Tyr Arg Pro Arg Ile Arg
225          230          235          240
Asn Ile Pro Ser Arg Ile Ser Ile Tyr Trp Thr Ile Val Lys Pro Gly
          245          250          255

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Asp Ile Leu Leu Ile Asn Ser Thr Gly Asn Leu Ile Ala Pro Arg Gly		
260	265	270
Tyr Phe Lys Ile Arg Ser Gly Lys Ser Ser Ile Met Arg Ser Asp Ala		
275	280	285
Pro Ile Gly Lys Cys Asn Ser Glu Cys Ile Thr Pro Asn Gly Ser Ile		
290	295	300
Pro Asn Asp Lys Pro Phe Gln Asn Val Asn Arg Ile Thr Tyr Gly Ala		
305	310	315
Cys Pro Arg Tyr Val Lys Gln Ser Thr Leu Lys Leu Ala Thr Gly Met		
325	330	335
Arg Asn Val Pro Glu Lys Gln Thr Arg Gly Ile Phe Gly Ala Ile Ala		
340	345	350
Gly Phe Ile Glu Asn Gly Trp Glu Gly Met Val Asp Gly Trp Tyr Gly		
355	360	365
Phe Arg His Gln Asn Ser Glu Gly Arg Gly Gln Ala Ala Asp Leu Lys		
370	375	380
Ser Thr Gln Ala Ala Ile Asp Gln Ile Asn Gly Lys Leu Asn Arg Leu		
385	390	395
Ile Gly Lys Thr Asn Glu Lys Phe His Gln Ile Glu Lys Glu Phe Ser		
405	410	415
Glu Val Glu Gly Arg Ile Gln Asp Leu Glu Lys Tyr Val Glu Asp Thr		
420	425	430
Lys Ile Asp Leu Trp Ser Tyr Asn Ala Glu Leu Leu Val Ala Leu Glu		
435	440	445
Asn Gln His Thr Ile Asp Leu Thr Asp Ser Glu Met Asn Lys Leu Phe		
450	455	460
Glu Lys Thr Lys Lys Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn		
465	470	475
Gly Cys Phe Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Gly Ser		
485	490	495
Ile Arg Asn Gly Thr Tyr Asp His Asp Val Tyr Arg Asp Glu Ala Leu		
500	505	510
Asn Asn Arg Phe Gln Ile Lys Ser Gly Gly Glu Ser Gln Val Arg Gln		
515	520	525
Gln Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys		
530	535	540
Glu Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr		
545	550	555
Thr His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala		

				565				570				575				
Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe	Leu	Asn	Glu	Asn	
				580				585				590				
Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	Ala	Pro	Glu	His	Lys	Phe	
				595				600				605				
Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr	Glu	His	Glu	Gln	His	
				610				615				620				
Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	His	Ala	Ile	Lys	Cys	Lys	
625				630				635				640				
Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr	Val	Ala	Glu	Gln	His	
				645				650				655				
Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp	Lys	Ile	Glu	Leu	Ile	
				660				665				670				
Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	Asp	Gln	Tyr	Val	Lys	Gly	
				675				680				685				
Ile	Ala	Lys	Ser	Arg	Lys	Ser										
690				695												
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Met	Glu	Lys	Ile	Val	Leu	Leu	Leu	Ala	Ile	Val	Ser	Leu	Val	Lys	Ser	
1	5				10				15							
Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Glu	Gln	Val	
20				25				30								
Asp	Thr	Ile	Met	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ala	Gln	Asp	Ile	
35				40				45								
Leu	Glu	Lys	Thr	His	Asn	Gly	Lys	Leu	Cys	Asp	Leu	Asp	Gly	Val	Lys	
50				55				60								
Pro	Leu	Ile	Leu	Arg	Asp	Cys	Ser	Val	Ala	Gly	Trp	Leu	Leu	Gly	Asn	
65				70				75				80				
Pro	Met	Cys	Asp	Glu	Phe	Ile	Asn	Val	Pro	Glu	Trp	Ser	Tyr	Ile	Val	
85				90				95								
Glu	Lys	Ala	Asn	Pro	Ala	Asn	Asp	Leu	Cys	Phe	Pro	Gly	Asn	Phe	Asn	

100	105	110
Asp Tyr Glu Glu Leu Lys His	Leu Leu Ser Arg Ile Asn His Phe Glu	
115	120	125
Lys Ile Gln Ile Ile Pro Lys	Ser Ser Trp Ser Asp His Glu Ala Ser	
130	135	140
Ser Gly Val Ser Ser Ala Cys	Pro Tyr Gln Gly Ser Pro Ser Phe Phe	
145	150	155
Arg Asn Val Val Trp Leu Ile	Lys Lys Asn Asn Thr Tyr Pro Thr Ile	
165	170	175
Lys Arg Ser Tyr Asn Asn Thr	Asn Gln Glu Asp Leu Leu Val Leu Trp	
180	185	190
Gly Ile His His Pro Asn Asp	Ala Ala Glu Gln Thr Arg Leu Tyr Gln	
195	200	205
Asn Pro Thr Thr Tyr Ile Ser	Val Gly Thr Ser Thr Leu Asn Gln Arg	
210	215	220
Leu Val Pro Lys Ile Ala Thr	Arg Ser Lys Val Asn Gly Gln Ser Gly	
225	230	235
Arg Met Glu Phe Phe Trp Thr	Ile Leu Lys Pro Asn Asp Ala Ile Asn	
245	250	255
Phe Glu Ser Asn Gly Asn Phe	Ile Ala Pro Glu Tyr Ala Tyr Lys Ile	
260	265	270
Val Lys Lys Gly Asp Ser Ala	Ile Met Lys Ser Glu Leu Glu Tyr Gly	
275	280	285
Asn Cys Asn Thr Lys Cys Gln	Thr Pro Ile Gly Ala Ile Asn Ser Ser	
290	295	300
Met Pro Phe His Asn Ile His	Pro Leu Thr Ile Gly Glu Cys Pro Lys	
305	310	315
Tyr Val Lys Ser Asn Arg Leu	Val Leu Ala Thr Gly Leu Arg Asn Ser	
325	330	335
Pro Gln Arg Glu Thr Arg Gly	Leu Phe Gly Ala Ile Ala Gly Phe Ile	
340	345	350
Glu Gly Gly Trp Gln Gly Met	Val Asp Gly Trp Tyr Gly Tyr His His	
355	360	365
Ser Asn Glu Gln Gly Ser Gly	Tyr Ala Ala Asp Lys Glu Ser Thr Gln	
370	375	380
Lys Ala Ile Asp Gly Val Thr	Asn Lys Val Asn Ser Ile Ile Asp Lys	
385	390	395
Met Asn Thr Gln Phe Glu Ala	Val Gly Arg Glu Phe Asn Asn Leu Glu	
405	410	415

Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp		
420	425	430
Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg		
435	440	445
Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val		
450	455	460
Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe		
465	470	475
Glu Phe Tyr His Lys Cys Asp Asn Glu Cys Met Glu Ser Val Arg Asn		
485	490	495
Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Lys Arg		
500	505	510
Glu Glu Ile Ser Ser Gly Gly Glu Ser Gln Val Arg Gln Gln Phe Ser		
515	520	525
Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln		
530	535	540
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser		
545	550	555
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr		
565	570	575
Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro		
580	585	590
Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu		
595	600	605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu		
610	615	620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp His Ala		
625	630	635
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu		
645	650	655
Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu		
660	665	670
Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys		
675	680	685
Ser Arg Lys Ser		
690		
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<211> 692		
<212> PRT		

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

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Met	Glu	Lys	Ile	Val	Leu	Leu	Leu	Ala	Ile	Val	Ser	Leu	Val	Lys	Ser
1				5					10					15	
Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Glu	Gln	Val
			20					25					30		
Asp	Thr	Ile	Met	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ala	Gln	Asp	Ile
		35					40					45			
Leu	Glu	Lys	Thr	His	Asn	Gly	Lys	Leu	Cys	Asp	Leu	Asp	Gly	Val	Lys
	50					55				60					
Pro	Leu	Ile	Leu	Arg	Asp	Cys	Ser	Val	Ala	Gly	Trp	Leu	Leu	Gly	Asn
65				70					75					80	
Pro	Met	Cys	Asp	Glu	Phe	Leu	Asn	Val	Pro	Glu	Trp	Ser	Tyr	Ile	Val
			85					90					95		
Glu	Lys	Ile	Asn	Pro	Ala	Asn	Asp	Leu	Cys	Phe	Pro	Gly	Asn	Phe	Asn
		100						105				110			
Asp	Tyr	Glu	Glu	Leu	Lys	His	Leu	Leu	Ser	Arg	Ile	Asn	His	Phe	Glu
	115					120					125				
Lys	Ile	Gln	Ile	Ile	Pro	Lys	Ser	Ser	Trp	Ser	Asp	His	Glu	Ala	Ser
	130					135					140				
Ser	Gly	Val	Ser	Ser	Ala	Cys	Pro	Tyr	Gln	Gly	Arg	Ser	Ser	Phe	Phe
145				150					155					160	
Arg	Asn	Val	Val	Trp	Leu	Ile	Lys	Lys	Asn	Asn	Ala	Tyr	Pro	Thr	Ile
		165						170				175			
Lys	Arg	Ser	Tyr	Asn	Asn	Thr	Asn	Gln	Glu	Asp	Leu	Leu	Val	Leu	Trp
		180					185				190				
Gly	Ile	His	His	Pro	Asn	Asp	Ala	Ala	Glu	Gln	Thr	Arg	Leu	Tyr	Gln
	195					200					205				
Asn	Pro	Thr	Thr	Tyr	Ile	Ser	Val	Gly	Thr	Ser	Thr	Leu	Asn	Gln	Arg
	210					215					220				
Leu	Val	Pro	Lys	Ile	Ala	Thr	Arg	Ser	Lys	Val	Asn	Gly	Gln	Ser	Gly
225				230					235					240	
Arg	Met	Glu	Phe	Phe	Trp	Thr	Ile	Leu	Lys	Pro	Asn	Asp	Ala	Ile	Asn
		245						250				255			
Phe	Glu	Ser	Asn	Gly	Asn	Phe	Ile	Ala	Pro	Glu	Asn	Ala	Tyr	Lys	Ile

260	265	270
Val Lys Lys Gly Asp Ser Thr Ile Met Lys Ser Glu Leu Glu Tyr Gly		
275	280	285
Asn Cys Asn Thr Lys Cys Gln Thr Pro Ile Gly Ala Ile Asn Ser Ser		
290	295	300
Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys		
305	310	315
Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Ser		
325	330	335
Pro Gln Ile Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile		
340	345	350
Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His		
355	360	365
Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln		
370	375	380
Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile Ile Asp Lys		
385	390	395
Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu Glu		
405	410	415
Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp		
420	425	430
Val Trp Thr Tyr Asn Ala Glu Leu Leu Val Leu Met Glu Asn Glu Arg		
435	440	445
Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val		
450	455	460
Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe		
465	470	475
Glu Phe Tyr His Arg Cys Asp Asn Glu Cys Met Glu Ser Val Arg Asn		
485	490	495
Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Lys Arg		
500	505	510
Glu Glu Ile Ser Ser Gly Gly Glu Ser Gln Val Arg Gln Gln Phe Ser		
515	520	525
Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln		
530	535	540
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser		
545	550	555
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr		
565	570	575

Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro
580 585 590
Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu
595 600 605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu
610 615 620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp His Ala
625 630 635 640
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu
645 650 655
Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu
660 665 670
Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys
675 680 685

Ser Arg Lys Ser
690

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<211> 692

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成
多肽"

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Met Glu Lys Ile Val Leu Leu Leu Ala Ile Val Ser Leu Val Lys Ser
1 5 10 15
Asp Gln Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Glu Gln Val
20 25 30
Asp Thr Ile Met Glu Lys Asn Val Thr Val Thr His Ala Gln Asp Ile
35 40 45
Leu Glu Lys Thr His Asn Gly Lys Leu Cys Asp Leu Asp Gly Val Lys
50 55 60
Pro Leu Ile Leu Arg Asp Cys Ser Val Ala Gly Trp Leu Leu Gly Asn
65 70 75 80
Pro Met Cys Asp Glu Phe Leu Asn Val Pro Glu Trp Ser Tyr Ile Val
85 90 95
Glu Lys Ile Asn Pro Ala Asn Asp Leu Cys Phe Pro Gly Asn Phe Asn
100 105 110

Asp Tyr Glu Glu Leu Lys His Leu Leu Ser Arg Ile Asn His Phe Glu		
115	120	125
Lys Ile Gln Ile Ile Pro Lys Ser Ser Trp Ser Asp His Glu Ala Ser		
130	135	140
Ser Gly Val Ser Ser Ala Cys Pro Tyr Gln Gly Arg Ser Ser Phe Phe		
145	150	155
Arg Asn Val Val Trp Leu Ile Lys Lys Asp Asn Ala Tyr Pro Thr Ile		
165	170	175
Lys Arg Ser Tyr Asn Asn Thr Asn Gln Glu Asp Leu Leu Val Leu Trp		
180	185	190
Gly Ile His His Pro Asn Asp Ala Ala Glu Gln Thr Arg Leu Tyr Gln		
195	200	205
Asn Pro Thr Thr Tyr Ile Ser Val Gly Thr Ser Thr Leu Asn Gln Arg		
210	215	220
Leu Val Pro Lys Ile Ala Thr Arg Ser Lys Val Asn Gly Gln Ser Gly		
225	230	235
Arg Met Glu Phe Phe Trp Thr Ile Leu Lys Pro Asn Asp Ala Ile Asn		
245	250	255
Phe Glu Ser Asn Gly Asn Phe Ile Ala Pro Glu Asn Ala Tyr Lys Ile		
260	265	270
Val Lys Lys Gly Asp Ser Thr Ile Met Lys Ser Glu Leu Glu Tyr Gly		
275	280	285
Asn Cys Asn Thr Lys Cys Gln Thr Pro Ile Gly Ala Ile Asn Ser Ser		
290	295	300
Met Pro Phe His Asn Ile His Pro Leu Thr Ile Gly Glu Cys Pro Lys		
305	310	315
Tyr Val Lys Ser Asn Arg Leu Val Leu Ala Thr Gly Leu Arg Asn Ser		
325	330	335
Pro Gln Gly Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile		
340	345	350
Glu Gly Gly Trp Gln Gly Met Val Asp Gly Trp Tyr Gly Tyr His His		
355	360	365
Ser Asn Glu Gln Gly Ser Gly Tyr Ala Ala Asp Lys Glu Ser Thr Gln		
370	375	380
Lys Ala Ile Asp Gly Val Thr Asn Lys Val Asn Ser Ile Ile Asp Lys		
385	390	395
Met Asn Thr Gln Phe Glu Ala Val Gly Arg Glu Phe Asn Asn Leu Glu		
405	410	415
Arg Arg Ile Glu Asn Leu Asn Lys Lys Met Glu Asp Gly Phe Leu Asp		

420	425	430
Val Trp Thr Tyr Asn Ala Glu Leu	Leu Val Leu Met Glu Asn Glu Arg	
435	440	445
Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr Asp Lys Val		
450	455	460
Arg Leu Gln Leu Arg Asp Asn Ala Lys Glu Leu Gly Asn Gly Cys Phe		
465	470	475
Glu Phe Tyr His Arg Cys Asp Asn Glu Cys Met Glu Ser Val Arg Asn		
485	490	495
Gly Thr Tyr Asp Tyr Pro Gln Tyr Ser Glu Glu Ala Arg Leu Lys Arg		
500	505	510
Glu Glu Ile Ser Ser Gly Gly Glu Ser Gln Val Arg Gln Gln Phe Ser		
515	520	525
Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln		
530	535	540
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser		
545	550	555
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr		
565	570	575
Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro		
580	585	590
Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu		
595	600	605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu		
610	615	620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp His Ala		
625	630	635
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu		
645	650	655
Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu		
660	665	670
Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys		
675	680	685
Ser Arg Lys Ser		
690		
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<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

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Met	Glu	Lys	Ile	Val	Leu	Leu	Leu	Ala	Ile	Val	Ser	Leu	Val	Lys	Ser	1	5	10	15
Asp	Gln	Ile	Cys	Ile	Gly	Tyr	His	Ala	Asn	Asn	Ser	Thr	Glu	Gln	Val	20	25	30	
Asp	Thr	Ile	Met	Glu	Lys	Asn	Val	Thr	Val	Thr	His	Ala	Gln	Asp	Ile	35	40	45	
Leu	Glu	Lys	Thr	His	Asn	Gly	Lys	Leu	Cys	Asp	Leu	Asp	Gly	Val	Lys	50	55	60	
Pro	Leu	Ile	Leu	Arg	Asp	Cys	Ser	Val	Ala	Gly	Trp	Leu	Leu	Gly	Asn	65	70	75	80
Pro	Met	Cys	Asp	Glu	Phe	Ile	Asn	Val	Pro	Glu	Trp	Ser	Tyr	Ile	Val	85	90	95	
Glu	Lys	Ala	Asn	Pro	Ala	Asn	Asp	Leu	Cys	Phe	Pro	Gly	Asn	Phe	Asn	100	105	110	
Asp	Tyr	Glu	Glu	Leu	Lys	His	Leu	Leu	Ser	Arg	Ile	Asn	His	Phe	Glu	115	120	125	
Lys	Ile	Gln	Ile	Ile	Pro	Lys	Ser	Ser	Trp	Ser	Asp	His	Glu	Ala	Ser	130	135	140	
Ser	Gly	Val	Ser	Ser	Ala	Cys	Pro	Tyr	Gln	Gly	Arg	Ser	Ser	Phe	Phe	145	150	155	160
Arg	Asn	Val	Val	Trp	Leu	Ile	Lys	Lys	Asn	Ser	Thr	Tyr	Pro	Thr	Ile	165	170	175	
Lys	Arg	Ser	Tyr	Asn	Asn	Thr	Asn	Gln	Glu	Asp	Leu	Leu	Val	Leu	Trp	180	185	190	
Gly	Ile	His	His	Pro	Asn	Asp	Ala	Ala	Glu	Gln	Thr	Arg	Leu	Tyr	Gln	195	200	205	
Asn	Pro	Thr	Thr	Tyr	Ile	Ser	Val	Gly	Thr	Ser	Thr	Leu	Asn	Gln	Arg	210	215	220	
Leu	Ala	Pro	Lys	Ile	Ala	Thr	Arg	Ser	Lys	Val	Asn	Gly	Gln	Ser	Gly	225	230	235	240
Arg	Met	Glu	Phe	Phe	Trp	Thr	Ile	Leu	Lys	Pro	Asn	Asp	Ala	Ile	Asn	245	250	255	
Phe	Glu	Ser	Asn	Gly	Asn	Phe	Ile	Ala	Pro	Glu	Tyr	Ala	Tyr	Lys	Ile	260	265	270	

Val	Lys	Lys	Gly	Asp	Ser	Ala	Ile	Met	Lys	Ser	Glu	Leu	Glu	Tyr	Gly	275	280	285
Asn	Cys	Asn	Thr	Lys	Cys	Gln	Thr	Pro	Met	Gly	Ala	Ile	Asn	Ser	Ser	290	295	300
Met	Pro	Phe	His	Asn	Ile	His	Pro	Leu	Thr	Ile	Gly	Glu	Cys	Pro	Lys	305	310	315
Tyr	Val	Lys	Ser	Asn	Arg	Leu	Val	Leu	Ala	Thr	Gly	Leu	Arg	Asn	Ser	325	330	335
Pro	Gln	Arg	Glu	Thr	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	Gly	Phe	Ile	340	345	350
Glu	Gly	Gly	Trp	Gln	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	Tyr	His	His	355	360	365
Ser	Asn	Glu	Gln	Gly	Ser	Gly	Tyr	Ala	Ala	Asp	Lys	Glu	Ser	Thr	Gln	370	375	380
Lys	Ala	Ile	Asp	Gly	Val	Thr	Asn	Lys	Val	Asn	Ser	Ile	Ile	Asp	Lys	385	390	395
Met	Asn	Thr	Gln	Phe	Glu	Ala	Val	Gly	Arg	Glu	Phe	Asn	Asn	Leu	Glu	405	410	415
Arg	Arg	Ile	Glu	Asn	Leu	Asn	Lys	Lys	Met	Glu	Asp	Gly	Phe	Leu	Asp	420	425	430
Val	Trp	Thr	Tyr	Asn	Ala	Glu	Leu	Leu	Val	Leu	Met	Glu	Asn	Glu	Arg	435	440	445
Thr	Leu	Asp	Phe	His	Asp	Ser	Asn	Val	Lys	Asn	Leu	Tyr	Asp	Lys	Val	450	455	460
Arg	Leu	Gln	Leu	Arg	Asp	Asn	Ala	Lys	Glu	Leu	Gly	Asn	Gly	Cys	Phe	465	470	475
Glu	Phe	Tyr	His	Arg	Cys	Asp	Asn	Glu	Cys	Met	Glu	Ser	Val	Arg	Asn	485	490	495
Gly	Thr	Tyr	Asp	Tyr	Pro	Gln	Tyr	Ser	Glu	Glu	Ala	Arg	Leu	Lys	Arg	500	505	510
Glu	Glu	Ile	Ser	Ser	Gly	Gly	Glu	Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser	515	520	525
Lys	Asp	Ile	Glu	Lys	Leu	Leu	Asn	Glu	Gln	Val	Asn	Lys	Glu	Met	Gln	530	535	540
Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met	Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser	545	550	555
Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu	Phe	Asp	His	Ala	Ala	Glu	Glu	Tyr	565	570	575
Glu	His	Ala	Lys	Lys	Leu	Ile	Ile	Phe	Leu	Asn	Glu	Asn	Asn	Val	Pro			

580	585	590
Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu		
595	600	605
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu		
610	615	620
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp His Ala		
625	630	635
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu		
645	650	655
Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu		
660	665	670
Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys		
675	680	685
Ser Arg Lys Ser		
690		
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<213> 人工序列		
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Thr Gly Asp Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His		
20 25 30		
Val Val Lys Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile		
35 40 45		
Pro Leu Thr Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly		
50 55 60		
Thr Glu Thr Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp		
65 70 75 80		
Leu Asp Val Ala Leu Gly Arg Pro Lys Cys Thr Gly Lys Ile Pro Ser		
85 90 95		
Ala Arg Val Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys		
100 105 110		
Phe Pro Ile Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu		

115	120	125
Leu Arg Gly Tyr Glu His Ile	Arg Leu Ser Thr His Asn Val Ile Asn	
130	135	140
Ala Glu Asn Ala Pro Gly Gly Pro Tyr Lys Ile Gly Thr Ser Gly Ser		
145	150	155
Cys Pro Asn Ile Thr Asn Gly Asn Gly Phe Phe Ala Thr Met Ala Trp		
165	170	175
Ala Val Pro Lys Asn Asp Lys Asn Lys Thr Ala Thr Asn Pro Leu Thr		
180	185	190
Ile Glu Val Pro Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val		
195	200	205
Trp Gly Phe His Ser Asp Asn Glu Thr Gln Met Ala Lys Leu Tyr Gly		
210	215	220
Asp Ser Lys Pro Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr		
225	230	235
His Tyr Val Ser Gln Ile Gly Gly Phe Pro Asn Gln Thr Glu Asp Gly		
245	250	255
Gly Leu Pro Gln Ser Gly Arg Ile Val Val Asp Tyr Met Val Gln Lys		
260	265	270
Ser Gly Lys Thr Gly Thr Ile Thr Tyr Gln Arg Gly Ile Leu Leu Pro		
275	280	285
Gln Lys Val Trp Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser		
290	295	300
Leu Pro Leu Ile Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly		
305	310	315
Leu Asn Lys Ser Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile		
325	330	335
Gly Asn Cys Pro Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly		
340	345	350
Thr Lys Tyr Arg Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe		
355	360	365
Gly Ala Ile Ala Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala		
370	375	380
Gly Trp His Gly Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala		
385	390	395
Ala Asp Leu Lys Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn		
405	410	415
Leu Asn Ser Leu Ser Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser		
420	425	430

Gly Ala Met Asp Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys		
435	440	445
Val Asp Asp Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala		
450	455	460
Val Leu Leu Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu		
465	470	475
Leu Ala Leu Glu Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val		
	485	490
Glu Ile Gly Asn Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr		
	500	505
Cys Leu Asp Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly Glu Phe Ser		
	515	520
Leu Pro Thr Phe Asp Ser Leu Asn Ile Thr Ala Ala Ser Ser Gly Arg		
	530	535
Ser Ser Gly Gly Asp Ile Ile Lys Leu Leu Asn Glu Gln Val Asn Lys		
545	550	555
Glu Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr		
	565	570
Thr His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala		
	580	585
Glu Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn		
	595	600
Asn Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe		
	610	615
Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His		
625	630	635
Ile Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys		
	645	650
Asp His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His		
	660	665
Glu Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile		
	675	680
Gly Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly		
	690	695
Ile Ala Lys Ser Arg Lys Ser Gly Ser		700
705	710	
<210> 37		
<211> 705		
<212> PRT		

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 37

Met	Lys	Ala	Ile	Ile	Val	Leu	Leu	Met	Val	Val	Thr	Ser	Asn	Ala	Asp
1				5					10					15	
Arg	Ile	Cys	Thr	Gly	Ile	Thr	Ser	Ser	Asn	Ser	Pro	His	Val	Val	Lys
			20					25					30		
Thr	Ala	Thr	Gln	Gly	Glu	Val	Asn	Val	Thr	Gly	Val	Ile	Pro	Leu	Thr
			35				40					45			
Thr	Thr	Pro	Thr	Lys	Ser	Tyr	Phe	Ala	Asn	Leu	Lys	Gly	Thr	Arg	Thr
	50					55				60					
Arg	Gly	Lys	Leu	Cys	Pro	Asp	Cys	Leu	Asn	Cys	Thr	Asp	Leu	Asp	Val
65				70					75					80	
Ala	Leu	Gly	Arg	Pro	Met	Cys	Val	Gly	Thr	Thr	Pro	Ser	Ala	Lys	Ala
				85					90					95	
Ser	Ile	Leu	His	Glu	Val	Arg	Pro	Val	Thr	Ser	Gly	Cys	Phe	Pro	Ile
			100					105					110		
Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Pro	Asn	Leu	Leu	Arg	Gly
	115					120					125				
Tyr	Glu	Lys	Ile	Arg	Leu	Ser	Thr	Gln	Asn	Val	Ile	Asp	Ala	Glu	Lys
	130					135					140				
Ala	Pro	Gly	Gly	Pro	Tyr	Arg	Leu	Gly	Thr	Ser	Gly	Ser	Cys	Pro	Asn
145				150					155					160	
Ala	Thr	Ser	Lys	Ile	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	Pro
			165						170					175	
Lys	Asp	Asn	Tyr	Lys	Asn	Ala	Thr	Asn	Pro	Leu	Thr	Val	Glu	Val	Pro
			180					185					190		
Tyr	Ile	Cys	Thr	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe	His
	195					200					205				
Ser	Asp	Asn	Lys	Thr	Gln	Met	Lys	Ser	Leu	Tyr	Gly	Asp	Ser	Asn	Pro
	210					215					220				
Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val	Ser
225				230					235					240	
Gln	Ile	Gly	Asp	Phe	Pro	Asp	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro	Gln
			245						250					255	
Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Met	Gln	Lys	Pro	Gly	Lys	Thr

260	265	270
Gly Thr Ile Val Tyr Gln Arg	Gly Val Leu Leu Pro Gln Lys Val Trp	
275	280	285
Cys Ala Ser Gly Arg Ser Lys	Val Ile Lys Gly Ser Leu Pro Leu Ile	
290	295	300
Gly Glu Ala Asp Cys Leu His	Glu Glu Tyr Gly Gly Leu Asn Lys Ser	
305	310	315
Lys Pro Tyr Tyr Thr Gly Lys	His Ala Lys Ala Ile Gly Asn Cys Pro	
325	330	335
Ile Trp Val Lys Thr Pro Leu	Lys Leu Ala Asn Gly Thr Lys Tyr Arg	
340	345	350
Pro Pro Ala Lys Leu Leu Lys	Glu Arg Gly Phe Phe Gly Ala Ile Ala	
355	360	365
Gly Phe Leu Glu Gly Gly Trp	Glu Gly Met Ile Ala Gly Trp His Gly	
370	375	380
Tyr Thr Ser His Gly Ala His	Gly Val Ala Val Ala Ala Asp Leu Lys	
385	390	395
Ser Thr Gln Glu Ala Ile Asn	Lys Ile Thr Lys Asn Leu Asn Ser Leu	
405	410	415
Ser Glu Leu Glu Val Lys Asn	Leu Gln Arg Leu Ser Gly Ala Met Asp	
420	425	430
Glu Leu His Asn Glu Ile Leu	Glu Leu Asp Glu Lys Val Asp Asp Leu	
435	440	445
Arg Ala Asp Thr Ile Ser Ser	Gln Ile Glu Leu Ala Val Leu Leu Ser	
450	455	460
Asn Glu Gly Ile Ile Asn Ser	Glu Asp Glu His Leu Leu Ala Leu Glu	
465	470	475
Arg Lys Leu Lys Lys Met Leu	Gly Pro Ser Ala Val Asp Ile Gly Asn	
485	490	495
Gly Cys Phe Glu Thr Lys His	Lys Cys Asn Gln Thr Cys Leu Asp Arg	
500	505	510
Ile Ala Ala Gly Thr Phe Asn	Ala Gly Glu Phe Ser Leu Pro Thr Phe	
515	520	525
Asp Ser Leu Asn Ile Thr Ala	Ala Ser Ser Gly Gly Asp Ile Ile Lys	
530	535	540
Leu Leu Asn Glu Gln Val Asn	Lys Glu Met Gln Ser Ser Asn Leu Tyr	
545	550	555
Met Ser Met Ser Ser Trp Ser	Tyr Thr His Ser Leu Asp Gly Ala Gly	
565	570	575

Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr Glu His Ala Lys Lys
 580 585 590
 Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro Val Gln Leu Thr Ser
 595 600 605
 Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu Thr Gln Ile Phe Gln
 610 615 620
 Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu Ser Ile Asn Asn Ile
 625 630 635 640
 Val Asp His Ala Ile Lys Cys Lys Asp His Ala Thr Phe Asn Phe Leu
 645 650 655
 Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu Val Leu Phe Lys Asp
 660 665 670
 Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu Asn His Gly Leu Tyr
 675 680 685
 Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys Ser Arg Lys Ser Gly
 690 695 700

Ser

705

<210> 38

<211> 701

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 38

Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp
 1 5 10 15
 Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
 20 25 30
 Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr
 35 40 45
 Thr Thr Pro Thr Lys Ser His Phe Ala Asn Leu Lys Gly Thr Glu Thr
 50 55 60
 Arg Gly Lys Leu Cys Pro Lys Cys Leu Asn Cys Thr Asp Leu Asp Val
 65 70 75 80
 Ala Leu Gly Arg Pro Lys Cys Thr Gly Lys Ile Pro Ser Ala Arg Val
 85 90 95

Ser	Ile	Leu	His	Glu	Val	Arg	Pro	Val	Thr	Ser	Gly	Cys	Phe	Pro	Ile	
Met	His	Asp	Arg	Thr	Lys	Ile	Arg	Gln	Leu	Pro	Asn	Leu	Leu	Arg	Gly	
Tyr	Glu	His	Ile	Arg	Leu	Ser	Thr	His	Asn	Val	Ile	Asn	Ala	Glu	Asn	
Ala	Pro	Gly	Gly	Pro	Tyr	Lys	Ile	Gly	Thr	Ser	Gly	Ser	Cys	Pro	Asn	
Ile	Thr	Asn	Gly	Asn	Gly	Phe	Phe	Ala	Thr	Met	Ala	Trp	Ala	Val	Pro	
Lys	Asn	Asp	Lys	Asn	Lys	Thr	Ala	Thr	Asn	Pro	Leu	Thr	Ile	Glu	Val	
Pro	Tyr	Ile	Cys	Thr	Glu	Gly	Glu	Asp	Gln	Ile	Thr	Val	Trp	Gly	Phe	
His	Ser	Asp	Asn	Glu	Thr	Gln	Met	Ala	Lys	Leu	Tyr	Gly	Asp	Ser	Lys	
Pro	Gln	Lys	Phe	Thr	Ser	Ser	Ala	Asn	Gly	Val	Thr	Thr	His	Tyr	Val	
Ser	Gln	Ile	Gly	Gly	Phe	Pro	Asn	Gln	Thr	Glu	Asp	Gly	Gly	Leu	Pro	
Gln	Ser	Gly	Arg	Ile	Val	Val	Asp	Tyr	Met	Val	Gln	Lys	Ser	Gly	Lys	
Thr	Gly	Thr	Ile	Thr	Tyr	Gln	Arg	Gly	Ile	Leu	Leu	Pro	Gln	Lys	Val	
Trp	Cys	Ala	Ser	Gly	Arg	Ser	Lys	Val	Ile	Lys	Gly	Ser	Leu	Pro	Leu	
Ile	Gly	Glu	Ala	Asp	Cys	Leu	His	Glu	Lys	Tyr	Gly	Gly	Leu	Asn	Lys	
Ser	Lys	Pro	Tyr	Tyr	Thr	Gly	Glu	His	Ala	Lys	Ala	Ile	Gly	Asn	Cys	
Pro	Ile	Trp	Val	Lys	Thr	Pro	Leu	Lys	Leu	Ala	Asn	Gly	Thr	Lys	Tyr	
Arg	Pro	Pro	Ala	Lys	Leu	Leu	Lys	Glu	Arg	Gly	Phe	Phe	Gly	Ala	Ile	
Ala	Gly	Phe	Leu	Glu	Gly	Gly	Trp	Glu	Gly	Met	Ile	Ala	Gly	Trp	His	
Gly	Tyr	Thr	Ser	His	Gly	Ala	His	Gly	Val	Ala	Val	Ala	Ala	Asp	Leu	
Lys	Ser	Thr	Gln	Glu	Ala	Ile	Asn	Lys	Ile	Thr	Lys	Asn	Leu	Asn	Ser	

	405		410		415
Leu Ser Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met					
	420		425		430
Asp Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp					
	435		440		445
Leu Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu					
	450		455		460
Ser Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu					
465		470		475	480
Glu Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Glu Ile Gly					
	485		490		495
Asn Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp					
	500		505		510
Arg Ile Ala Ala Gly Thr Phe Asp Ala Gly Glu Phe Ser Leu Pro Thr					
	515		520		525
Phe Asp Ser Leu Asn Ile Thr Ser Gly Gly Asp Ile Ile Lys Leu Leu					
	530		535		540
Asn Glu Gln Val Asn Lys Glu Met Gln Ser Ser Asn Leu Tyr Met Ser					
545		550		555	560
Met Ser Ser Trp Ser Tyr Thr His Ser Leu Asp Gly Ala Gly Leu Phe					
	565		570		575
Leu Phe Asp His Ala Ala Glu Glu Tyr Glu His Ala Lys Lys Leu Ile					
	580		585		590
Ile Phe Leu Asn Glu Asn Asn Val Pro Val Gln Leu Thr Ser Ile Ser					
	595		600		605
Ala Pro Glu His Lys Phe Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala					
	610		615		620
Tyr Glu His Glu Gln His Ile Ser Glu Ser Ile Asn Asn Ile Val Asp					
625		630		635	640
His Ala Ile Lys Cys Lys Asp His Ala Thr Phe Asn Phe Leu Gln Trp					
	645		650		655
Tyr Val Ala Glu Gln His Glu Glu Glu Val Leu Phe Lys Asp Ile Leu					
	660		665		670
Asp Lys Ile Glu Leu Ile Gly Asn Glu Asn His Gly Leu Tyr Leu Ala					
	675		680		685
Asp Gln Tyr Val Lys Gly Ile Ala Lys Ser Arg Lys Ser					
	690		695		700

<210> 39

<211> 703

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 39

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Met Lys Ala Ile Ile Val Leu Leu Met Val Val Thr Ser Asn Ala Asp
1           5           10           15
Arg Ile Cys Thr Gly Ile Thr Ser Ser Asn Ser Pro His Val Val Lys
          20           25           30
Thr Ala Thr Gln Gly Glu Val Asn Val Thr Gly Val Ile Pro Leu Thr
          35           40           45
Thr Thr Pro Thr Lys Ser Tyr Phe Ala Asn Leu Lys Gly Thr Arg Thr
          50           55           60
Arg Gly Lys Leu Cys Pro Asp Cys Leu Asn Cys Thr Asp Leu Asp Val
65           70           75           80
Ala Leu Gly Arg Pro Met Cys Val Gly Thr Thr Pro Ser Ala Lys Ala
          85           90           95
Ser Ile Leu His Glu Val Arg Pro Val Thr Ser Gly Cys Phe Pro Ile
          100          105          110
Met His Asp Arg Thr Lys Ile Arg Gln Leu Pro Asn Leu Leu Arg Gly
          115          120          125
Tyr Glu Asn Ile Arg Leu Ser Thr Gln Asn Val Ile Asp Ala Glu Lys
          130          135          140
Ala Pro Gly Gly Pro Tyr Arg Leu Gly Thr Ser Gly Ser Cys Pro Asn
145          150          155          160
Ala Thr Ser Lys Ile Gly Phe Phe Ala Thr Met Ala Trp Ala Val Pro
          165          170          175
Lys Asp Asn Tyr Lys Asn Ala Thr Asn Pro Leu Thr Val Glu Val Pro
          180          185          190
Tyr Ile Cys Thr Glu Gly Glu Asp Gln Ile Thr Val Trp Gly Phe His
          195          200          205
Ser Asp Asn Lys Thr Gln Met Lys Ser Leu Tyr Gly Asp Ser Asn Pro
          210          215          220
Gln Lys Phe Thr Ser Ser Ala Asn Gly Val Thr Thr His Tyr Val Ser
225          230          235          240
Gln Ile Gly Asp Phe Pro Asp Gln Thr Glu Asp Gly Gly Leu Pro Gln
          245          250          255

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Ser Gly Arg Ile Val Val Asp Tyr Met Met Gln Lys Pro Gly Lys Thr			
260	265	270	
Gly Thr Ile Val Tyr Gln Arg Gly Val Leu Leu Pro Gln Lys Val Trp			
275	280	285	
Cys Ala Ser Gly Arg Ser Lys Val Ile Lys Gly Ser Leu Pro Leu Ile			
290	295	300	
Gly Glu Ala Asp Cys Leu His Glu Lys Tyr Gly Gly Leu Asn Lys Ser			
305	310	315	320
Lys Pro Tyr Tyr Thr Gly Glu His Ala Lys Ala Ile Gly Asn Cys Pro			
325	330	335	
Ile Trp Val Lys Thr Pro Leu Lys Leu Ala Asn Gly Thr Lys Tyr Arg			
340	345	350	
Pro Pro Ala Lys Leu Leu Lys Glu Arg Gly Phe Phe Gly Ala Ile Ala			
355	360	365	
Gly Phe Leu Glu Gly Gly Trp Glu Gly Met Ile Ala Gly Trp His Gly			
370	375	380	
Tyr Thr Ser His Gly Ala His Gly Val Ala Val Ala Ala Asp Leu Lys			
385	390	395	400
Ser Thr Gln Glu Ala Ile Asn Lys Ile Thr Lys Asn Leu Asn Ser Leu			
405	410	415	
Ser Glu Leu Glu Val Lys Asn Leu Gln Arg Leu Ser Gly Ala Met Asp			
420	425	430	
Glu Leu His Asn Glu Ile Leu Glu Leu Asp Glu Lys Val Asp Asp Leu			
435	440	445	
Arg Ala Asp Thr Ile Ser Ser Gln Ile Glu Leu Ala Val Leu Leu Ser			
450	455	460	
Asn Glu Gly Ile Ile Asn Ser Glu Asp Glu His Leu Leu Ala Leu Glu			
465	470	475	480
Arg Lys Leu Lys Lys Met Leu Gly Pro Ser Ala Val Asp Ile Gly Asn			
485	490	495	
Gly Cys Phe Glu Thr Lys His Lys Cys Asn Gln Thr Cys Leu Asp Arg			
500	505	510	
Ile Ala Ala Gly Thr Phe Asn Ala Gly Glu Phe Ser Leu Pro Thr Phe			
515	520	525	
Asp Ser Leu Asn Ile Thr Ala Ser Gly Gly Asp Ile Ile Lys Leu Leu			
530	535	540	
Asn Glu Gln Val Asn Lys Glu Met Gln Ser Ser Asn Leu Tyr Met Ser			
545	550	555	560
Met Ser Ser Trp Ser Tyr Thr His Ser Leu Asp Gly Ala Gly Leu Phe			

	565		570		575
Leu Phe Asp His Ala Ala Glu Glu Tyr Glu His Ala Lys Lys Leu Ile					
	580		585		590
Ile Phe Leu Asn Glu Asn Asn Val Pro Val Gln Leu Thr Ser Ile Ser					
	595		600		605
Ala Pro Glu His Lys Phe Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala					
	610		615		620
Tyr Glu His Glu Gln His Ile Ser Glu Ser Ile Asn Asn Ile Val Asp					
625		630		635	640
His Ala Ile Lys Cys Lys Asp His Ala Thr Phe Asn Phe Leu Gln Trp					
	645		650		655
Tyr Val Ala Glu Gln His Glu Glu Glu Val Leu Phe Lys Asp Ile Leu					
	660		665		670
Asp Lys Ile Glu Leu Ile Gly Asn Glu Asn His Gly Leu Tyr Leu Ala					
	675		680		685
Asp Gln Tyr Val Lys Gly Ile Ala Lys Ser Arg Lys Ser Gly Ser					
	690		695		700
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<221> 来源					
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Met Lys Thr Ile Ile Ala Leu Ser Tyr Ile Leu Cys Leu Val Phe Ala					
1	5		10		15
Gln Lys Leu Pro Gly Asn Asp Asn Ser Thr Ala Thr Leu Cys Leu Gly					
	20		25		30
His His Ala Val Pro Asn Gly Thr Ile Val Lys Thr Ile Thr Asn Asp					
	35		40		45
Gln Ile Glu Val Thr Asn Ala Thr Glu Leu Val Phe Pro Gly Cys Gly					
	50		55		60
Val Leu Lys Leu Ala Thr Gly Met Arg Asn Val Pro Glu Lys Gln Thr					
65		70		75	80
Arg Gly Ile Phe Gly Ala Ile Ala Gly Phe Ile Glu Asn Gly Trp Glu					
	85		90		95
Gly Met Val Asp Gly Trp Tyr Gly Phe Arg His Gln Asn Ser Glu Gly					

100	105	110
Ile Gly Gln Ala Ala Asp Leu Lys Ser Thr Gln Ala Ala Ile Asn Gln		
115	120	125
Ile Asn Gly Met Val Asn Arg Val Ile Glu Leu Met Glu Gln Gly Gly		
130	135	140
Pro Asp Cys Tyr Leu Ala Glu Leu Leu Val Ala Leu Leu Asn Gln His		
145	150	155
Val Ile Asp Leu Thr Asp Ser Glu Met Arg Lys Leu Phe Glu Arg Thr		
165	170	175
Lys Lys Gln Leu Arg Glu Asn Ala Glu Asp Met Gly Asn Gly Cys Phe		
180	185	190
Lys Ile Tyr His Lys Cys Asp Asn Ala Cys Ile Gly Ser Ile Arg Asn		
195	200	205
Gly Thr Tyr Asp His Asp Val Tyr Arg Asp Glu Ala Leu Asn Asn Arg		
210	215	220
Phe Gln Ile Lys Ser Gly Gly Glu Ser Gln Val Arg Gln Gln Phe Ser		
225	230	235
Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys Glu Met Gln		
245	250	255
Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr Thr His Ser		
260	265	270
Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala Glu Glu Tyr		
275	280	285
Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn Asn Val Pro		
290	295	300
Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe Glu Gly Leu		
305	310	315
Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His Ile Ser Glu		
325	330	335
Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys Asp His Ala		
340	345	350
Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His Glu Glu Glu		
355	360	365
Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile Gly Asn Glu		
370	375	380
Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly Ile Ala Lys		
385	390	395
Ser Arg Lys Ser		400

<210> 41

<211> 397

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 41

Met	Asn	Thr	Gln	Ile	Leu	Val	Phe	Ala	Leu	Ile	Ala	Ile	Ile	Pro	Thr
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Asn	Ala	Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ser	Asn	Gly	Thr
			20					25					30		
Lys	Val	Asn	Thr	Leu	Thr	Glu	Arg	Gly	Val	Glu	Val	Val	Asn	Ala	Thr
			35					40					45		
Glu	Leu	Val	Phe	Pro	Gly	Cys	Gly	Val	Leu	Lys	Leu	Ala	Thr	Gly	Met
			50				55				60				
Lys	Asn	Val	Pro	Glu	Ile	Pro	Lys	Gly	Arg	Gly	Leu	Phe	Gly	Ala	Ile
65					70					75					80
Ala	Gly	Phe	Ile	Glu	Asn	Gly	Trp	Glu	Gly	Leu	Ile	Asp	Gly	Trp	Tyr
					85					90				95	
Gly	Phe	Arg	His	Gln	Asn	Ala	Gln	Gly	Glu	Gly	Thr	Ala	Ala	Asp	Tyr
					100					105				110	
Lys	Ser	Thr	Gln	Ser	Ala	Ile	Asp	Gln	Ile	Thr	Gly	Met	Val	Asn	Arg
					115					120				125	
Val	Ile	Glu	Leu	Met	Glu	Gln	Gly	Gly	Pro	Asp	Cys	Tyr	Leu	Ala	Glu
							135				140				
Leu	Leu	Val	Ala	Met	Leu	Asn	Gln	His	Val	Ile	Asp	Leu	Ala	Asp	Ser
145						150					155				160
Glu	Met	Asp	Lys	Leu	Tyr	Glu	Arg	Val	Lys	Arg	Gln	Leu	Arg	Glu	Asn
						165					170			175	
Ala	Glu	Glu	Asp	Gly	Thr	Gly	Cys	Phe	Glu	Ile	Phe	His	Lys	Cys	Asp
					180						185			190	
Asp	Asp	Cys	Met	Ala	Ser	Ile	Arg	Asn	Asn	Thr	Tyr	Asp	His	Ser	Lys
					195					200				205	
Tyr	Arg	Glu	Glu	Ala	Met	Gln	Asn	Arg	Ile	Gln	Ile	Asp	Ser	Gly	Gly
					210					215				220	
Glu	Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu
225						230					235				240
Asn	Glu	Gln	Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser

				245				250				255				
Met	Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	
				260				265				270				
Leu	Phe	Asp	His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	
				275				280				285				
Ile	Phe	Leu	Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	
				290				295				300				
Ala	Pro	Glu	His	Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	
305				310				315				320				
Tyr	Glu	His	Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	
				325				330				335				
His	Ala	Ile	Lys	Cys	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	
				340				345				350				
Tyr	Val	Ala	Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	
				355				360				365				
Asp	Lys	Ile	Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	
				370				375				380				
Asp	Gln	Tyr	Val	Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser				
385				390				395								
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<223> /注释="人工序列的描述：合成多肽"																
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Leu	Asp	Lys	Ile	Cys	Leu	Gly	His	His	Ala	Val	Ala	Asn	Gly	Thr	Ile	
				20					25				30			
Val	Lys	Thr	Leu	Thr	Asn	Glu	Gln	Glu	Glu	Val	Thr	Asn	Ala	Thr	Glu	
				35					40				45			
Leu	Val	Phe	Pro	Gly	Cys	Gly	Val	Leu	Met	Leu	Ala	Thr	Gly	Met	Arg	
				50					55				60			
Asn	Val	Pro	Glu	Leu	Ile	Gln	Gly	Arg	Gly	Leu	Phe	Gly	Ala	Ile	Ala	
65				70				75				80				
Gly	Phe	Leu	Glu	Asn	Gly	Trp	Glu	Gly	Met	Val	Asp	Gly	Trp	Tyr	Gly	

				85					90					95			
Phe	Arg	His	Gln	Asn	Ala	Gln	Gly	Thr	Gly	Gln	Ala	Ala	Asp	Tyr	Lys		
				100					105					110			
Ser	Thr	Gln	Ala	Ala	Ile	Asp	Gln	Ile	Thr	Gly	Met	Val	Asn	Arg	Val		
				115					120					125			
Val	Glu	Leu	Met	Glu	Gln	Gly	Gly	Pro	Asp	Cys	Tyr	Leu	Ala	Glu	Leu		
				130					135					140			
Leu	Val	Ala	Met	Leu	Asn	Gln	His	Val	Ile	Asp	Met	Ala	Asp	Ser	Glu		
145						150					155				160		
Met	Arg	Asn	Leu	Tyr	Glu	Arg	Val	Arg	Lys	Gln	Leu	Arg	Gln	Asn	Ala		
				165					170					175			
Glu	Glu	Asp	Gly	Lys	Gly	Cys	Phe	Glu	Ile	Tyr	His	Ala	Cys	Asp	Asp		
				180					185					190			
Ser	Cys	Met	Glu	Ser	Ile	Arg	Asn	Asn	Thr	Tyr	Asp	His	Ser	Gln	Tyr		
				195					200					205			
Arg	Glu	Glu	Ala	Leu	Leu	Asn	Arg	Leu	Asn	Ile	Asn	Ser	Gly	Gly	Glu		
				210					215					220			
Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu	Asn		
225						230				235					240		
Glu	Gln	Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser	Met		
				245						250				255			
Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	Leu		
				260					265					270			
Phe	Asp	His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	Ile		
				275					280					285			
Phe	Leu	Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	Ala		
				290					295					300			
Pro	Glu	His	Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	Tyr		
305						310				315					320		
Glu	His	Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	His		
				325						330				335			
Ala	Ile	Lys	Cys	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	Tyr		
				340					345					350			
Val	Ala	Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	Asp		
				355					360					365			
Lys	Ile	Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	Asp		
				370					375					380			
Gln	Tyr	Val	Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser						
385						390								395			

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<213> 人工序列

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<221> 来源

<223> /注释="人工序列的描述：合成多肽"

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Met Lys Ala Lys Leu Leu Val Leu Leu Cys Thr Phe Thr Ala Thr Tyr
1           5           10           15
Ala Asp Thr Ile Cys Ile Gly Tyr His Ala Asn Asn Ser Thr Asp Thr
           20           25           30
Val Asp Thr Val Leu Glu Lys Asn Val Thr Val Thr His Ser Val Asn
           35           40           45
Leu Gly Ser Gly Leu Arg Met Val Thr Gly Leu Arg Asn Ile Pro Gln
           50           55           60
Arg Glu Thr Arg Gly Leu Phe Gly Ala Ile Ala Gly Phe Ile Glu Gly
65           70           75           80
Gly Trp Thr Gly Met Val Asp Gly Trp Tyr Gly Tyr His His Gln Asn
           85           90           95
Glu Gln Gly Ser Gly Tyr Ala Ala Asp Gln Lys Ser Thr Gln Asn Ala
           100          105          110
Ile Asn Gly Ile Thr Asn Met Val Asn Ser Val Ile Glu Lys Met Gly
           115          120          125
Ser Gly Gly Ser Gly Thr Asp Leu Ala Glu Leu Leu Val Leu Leu Leu
           130          135          140
Asn Glu Arg Thr Leu Asp Phe His Asp Ser Asn Val Lys Asn Leu Tyr
145          150          155          160
Glu Lys Val Lys Ser Gln Leu Lys Asn Asn Ala Lys Glu Ile Gly Asn
           165          170          175
Gly Cys Phe Glu Phe Tyr His Lys Cys Asn Asn Glu Cys Met Glu Ser
           180          185          190
Val Lys Asn Gly Thr Tyr Asp Tyr Pro Lys Tyr Ser Glu Glu Ser Lys
           195          200          205
Leu Asn Arg Glu Lys Ile Asp Ser Gly Gly Glu Ser Gln Val Arg Gln
           210          215          220
Gln Phe Ser Lys Asp Ile Glu Lys Leu Leu Asn Glu Gln Val Asn Lys
225          230          235          240

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Glu Met Gln Ser Ser Asn Leu Tyr Met Ser Met Ser Ser Trp Ser Tyr		
245	250	255
Thr His Ser Leu Asp Gly Ala Gly Leu Phe Leu Phe Asp His Ala Ala		
260	265	270
Glu Glu Tyr Glu His Ala Lys Lys Leu Ile Ile Phe Leu Asn Glu Asn		
275	280	285
Asn Val Pro Val Gln Leu Thr Ser Ile Ser Ala Pro Glu His Lys Phe		
290	295	300
Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala Tyr Glu His Glu Gln His		
305	310	315
Ile Ser Glu Ser Ile Asn Asn Ile Val Asp His Ala Ile Lys Cys Lys		
325	330	335
Asp His Ala Thr Phe Asn Phe Leu Gln Trp Tyr Val Ala Glu Gln His		
340	345	350
Glu Glu Glu Val Leu Phe Lys Asp Ile Leu Asp Lys Ile Glu Leu Ile		
355	360	365
Gly Asn Glu Asn His Gly Leu Tyr Leu Ala Asp Gln Tyr Val Lys Gly		
370	375	380
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<223> /注释="人工序列的描述：合成
多肽"

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Glu	Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu
1				5					10					15	
Asn	Glu	Gln	Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Cys
			20					25					30		
Met	Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe
			35				40					45			
Leu	Phe	Asp	His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile
			50				55				60				
Ile	Phe	Leu	Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser
65					70					75				80	
Ala	Pro	Glu	His	Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala
				85					90					95	
Tyr	Glu	His	Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp
				100					105				110		
His	Ala	Ile	Lys	Ser	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp
			115					120					125		
Tyr	Val	Ala	Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu
			130				135				140				
Asp	Lys	Ile	Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala
145					150					155				160	
Asp	Gln	Tyr	Val	Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser			
				165					170						

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<223> /注释="人工序列的描述：合成
多肽"

<400> 202

Glu	Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu
1				5					10					15	
Asn	Glu	Gln	Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser
			20					25					30		

Met	Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	
35				40				45								
Leu	Phe	Asp	His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	
50				55				60								
Ile	Phe	Leu	Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Cys	Ile	Ser	
65				70				75				80				
Ala	Pro	Glu	His	Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	
				85				90				95				
Tyr	Glu	His	Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	
				100				105				110				
His	Ala	Ile	Lys	Ser	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	
115				120				125								
Tyr	Val	Ala	Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	
130				135				140								
Asp	Lys	Ile	Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	
145				150				155				160				
Asp	Gln	Tyr	Val	Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser				
				165				170								

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<213> 人工序列

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<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 203

Glu	Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu
1	5				10				15						
Asn	Glu	Gln	Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser
20				25				30							
Met	Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe
35				40				45							
Leu	Phe	Asp	His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile
50				55				60							
Ile	Phe	Leu	Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser
65				70				75				80			
Cys	Pro	Glu	His	Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala
				85				90				95			

Tyr	Glu	His	Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	
100					105					110						
His	Ala	Ile	Lys	Ser	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	
115					120					125						
Tyr	Val	Ala	Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	
130					135					140						
Asp	Lys	Ile	Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	
145					150					155					160	
Asp	Gln	Tyr	Val	Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser				
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<223> /注释="人工序列的描述：合成多肽"

<400> 204

Glu	Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu	
1				5				10				15				
Asn	Glu	Gln	Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser	
20					25					30						
Met	Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe	
35					40					45						
Leu	Phe	Asp	His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile	
50					55					60						
Ile	Phe	Leu	Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser	
65					70					75					80	
Ala	Pro	Glu	His	Cys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala	
85					90					95						
Tyr	Glu	His	Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp	
100					105					110						
His	Ala	Ile	Lys	Ser	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp	
115					120					125						
Tyr	Val	Ala	Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu	
130					135					140						
Asp	Lys	Ile	Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala	
145					150					155					160	

Asp Gln Tyr Val Lys Gly Ile Ala Lys Ser Arg Lys Ser
165 170

<210> 205

<211> 173

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成
多肽"

<400> 205

Glu Ser Gln Val Arg Gln Gln Phe Ser Lys Asp Ile Glu Lys Leu Leu
1 5 10 15
Asn Glu Gln Val Asn Lys Glu Met Gln Ser Ser Asn Leu Tyr Met Ser
20 25 30
Met Ser Ser Trp Ser Tyr Thr His Ser Leu Asp Gly Ala Gly Leu Phe
35 40 45
Leu Phe Asp His Ala Ala Glu Glu Tyr Glu His Ala Lys Lys Leu Ile
50 55 60
Ile Phe Leu Asn Glu Asn Asn Val Pro Val Gln Leu Thr Ser Ile Ser
65 70 75 80
Ala Pro Glu His Lys Phe Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala
85 90 95
Tyr Glu His Glu Gln His Ile Ser Glu Cys Ile Asn Asn Ile Val Asp
100 105 110
His Ala Ile Lys Ser Lys Asp His Ala Thr Phe Asn Phe Leu Gln Trp
115 120 125
Tyr Val Ala Glu Gln His Glu Glu Glu Val Leu Phe Lys Asp Ile Leu
130 135 140
Asp Lys Ile Glu Leu Ile Gly Asn Glu Asn His Gly Leu Tyr Leu Ala
145 150 155 160
Asp Gln Tyr Val Lys Gly Ile Ala Lys Ser Arg Lys Ser
165 170

<210> 206

<211> 173

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 206

Glu	Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu
1				5					10					15	
Asn	Glu	Gln	Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser
				20				25						30	
Met	Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe
				35				40						45	
Leu	Phe	Asp	His	Ala	Ala	Glu	Glu	Tyr	Glu	His	Ala	Lys	Lys	Leu	Ile
				50				55						60	
Ile	Phe	Leu	Asn	Glu	Asn	Asn	Val	Pro	Val	Gln	Leu	Thr	Ser	Ile	Ser
65							70				75				80
Ala	Pro	Glu	His	Lys	Phe	Glu	Gly	Leu	Thr	Gln	Ile	Phe	Gln	Lys	Ala
				85						90					95
Tyr	Glu	His	Glu	Gln	His	Ile	Ser	Glu	Ser	Ile	Asn	Asn	Ile	Val	Asp
				100						105				110	
His	Ala	Ile	Lys	Cys	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp
				115						120				125	
Tyr	Val	Ala	Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu
				130						135				140	
Asp	Lys	Ile	Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala
145							150				155				160
Asp	Gln	Tyr	Val	Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser			
				165							170				

<210> 207

<211> 173

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 207

Glu	Ser	Gln	Val	Arg	Gln	Gln	Phe	Ser	Lys	Asp	Ile	Glu	Lys	Leu	Leu
1				5					10					15	
Asn	Cys	Gln	Val	Asn	Lys	Glu	Met	Gln	Ser	Ser	Asn	Leu	Tyr	Met	Ser
				20					25					30	
Met	Ser	Ser	Trp	Ser	Tyr	Thr	His	Ser	Leu	Asp	Gly	Ala	Gly	Leu	Phe

35	40	45
Leu Phe Asp His Ala Ala Glu Glu Tyr Glu His Ala Lys Lys Leu Ile		
50	55	60
Ile Phe Leu Asn Glu Asn Asn Val Pro Val Gln Leu Thr Ser Ile Ser		
65	70	75
Ala Pro Glu His Lys Phe Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala		
85	90	95
Tyr Glu His Glu Gln His Ile Ser Glu Ser Ile Asn Asn Ile Val Asp		
100	105	110
His Ala Ile Lys Ser Lys Asp His Ala Thr Phe Asn Phe Leu Gln Trp		
115	120	125
Tyr Val Ala Glu Gln His Glu Glu Glu Val Leu Phe Lys Asp Ile Leu		
130	135	140
Asp Lys Ile Glu Leu Ile Gly Asn Glu Asn His Gly Leu Tyr Leu Ala		
145	150	155
Asp Gln Tyr Val Lys Gly Ile Ala Lys Ser Arg Lys Ser		
165	170	

<210> 208

<211> 173

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 208

Glu Ser Gln Val Arg Gln Gln Phe Ser Lys Asp Ile Glu Lys Leu Leu		
1	5	10
Asn Glu Gln Val Asn Lys Glu Met Asn Ser Ser Asn Leu Tyr Met Ser		
20	25	30
Met Ser Ser Trp Cys Tyr Thr His Ser Leu Asp Gly Ala Gly Leu Phe		
35	40	45
Leu Phe Asp His Ala Ala Glu Glu Tyr Glu His Ala Lys Lys Leu Ile		
50	55	60
Ile Phe Leu Asn Glu Asn Asn Val Pro Val Gln Leu Thr Ser Ile Ser		
65	70	75
Ala Pro Glu His Lys Phe Glu Gly Leu Thr Gln Ile Phe Gln Lys Ala		
85	90	95
Tyr Glu His Glu Gln His Ile Ser Glu Ser Ile Asn Asn Ile Val Asp		

100						105						110					
His	Ala	Ile	Lys	Ser	Lys	Asp	His	Ala	Thr	Phe	Asn	Phe	Leu	Gln	Trp		
115						120						125					
Tyr	Val	Ala	Glu	Gln	His	Glu	Glu	Glu	Val	Leu	Phe	Lys	Asp	Ile	Leu		
130						135						140					
Asp	Lys	Ile	Glu	Leu	Ile	Gly	Asn	Glu	Asn	His	Gly	Leu	Tyr	Leu	Ala		
145						150						155					
Asp	Gln	Tyr	Val	Lys	Gly	Ile	Ala	Lys	Ser	Arg	Lys	Ser					
165						170											

<210> 209

⟨211⟩ 166

<212> PRT

〈213〉 幽门螺杆菌 (*Helicobacter pylori*)

<400> 209

[illegible] $\langle 210 \rangle$ 210

211 22

⟨212⟩ DNA

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成寡核苷酸"

<400> 210

tgactgtgaa cgttcgagat ga 22

<210> 211

<211> 191

<212> PRT

<213> 粉纹夜蛾(Trichoplusia ni)

<400> 211

Thr Gln Cys Asn Val Asn Pro Val Gln Ile Pro Lys Asp Trp Ile Thr

1 5 10 15

Met His Arg Ser Cys Arg Asn Ser Met Arg Gln Gln Ile Gln Met Glu

20 25 30

Val Gly Ala Ser Leu Gln Tyr Leu Ala Met Gly Ala His Phe Ser Lys

35 40 45

Asp Val Val Asn Arg Pro Gly Phe Ala Gln Leu Phe Phe Asp Ala Ala

50 55 60

Ser Glu Glu Arg Glu His Ala Met Lys Leu Ile Glu Tyr Leu Leu Met

65 70 75 80

Arg Gly Glu Leu Thr Asn Asp Val Ser Ser Leu Leu Gln Val Arg Pro

85 90 95

Pro Thr Arg Ser Ser Trp Lys Gly Gly Val Glu Ala Leu Glu His Ala

100 105 110

Leu Ser Met Glu Ser Asp Val Thr Lys Ser Ile Arg Asn Val Ile Lys

115 120 125

Ala Cys Glu Asp Asp Ser Glu Phe Asn Asp Tyr His Leu Val Asp Tyr

130 135 140

Leu Thr Gly Asp Phe Leu Glu Glu Gln Tyr Lys Gly Gln Arg Asp Leu

145 150 155 160

Ala Gly Lys Ala Ser Thr Leu Lys Lys Leu Met Asp Arg His Glu Ala

165 170 175

Leu Gly Glu Phe Ile Phe Asp Lys Lys Leu Leu Gly Ile Asp Val

180 185 190

<210> 212

<211> 212

<212> PRT

<213> 粉纹夜蛾(*Trichoplusia ni*)

<400> 212

Ala	Asp	Thr	Cys	Tyr	Asn	Asp	Val	Ala	Leu	Asp	Cys	Gly	Ile	Thr	Ser
1				5					10					15	
Asn	Ser	Leu	Ala	Leu	Pro	Arg	Cys	Asn	Ala	Val	Tyr	Gly	Glu	Tyr	Gly
		20					25						30		
Ser	His	Gly	Asn	Val	Ala	Thr	Glu	Leu	Gln	Ala	Tyr	Ala	Lys	Leu	His
		35					40					45			
Leu	Glu	Arg	Ser	Tyr	Asp	Tyr	Leu	Leu	Ser	Ala	Ala	Tyr	Phe	Asn	Asn
	50					55				60					
Tyr	Gln	Thr	Asn	Arg	Ala	Gly	Phe	Ser	Lys	Leu	Phe	Lys	Lys	Leu	Ser
65				70					75					80	
Asp	Glu	Ala	Trp	Ser	Lys	Thr	Ile	Asp	Ile	Ile	Lys	His	Val	Thr	Lys
			85					90					95		
Arg	Gly	Asp	Lys	Met	Asn	Phe	Asp	Gln	His	Ser	Thr	Met	Lys	Thr	Glu
			100					105					110		
Arg	Lys	Asn	Tyr	Thr	Ala	Glu	Asn	His	Glu	Leu	Glu	Ala	Leu	Ala	Lys
		115						120					125		
Ala	Leu	Asp	Thr	Gln	Lys	Glu	Leu	Ala	Glu	Arg	Ala	Phe	Tyr	Ile	His
		130					135					140			
Arg	Glu	Ala	Thr	Arg	Asn	Ser	Gln	His	Leu	His	Asp	Pro	Glu	Ile	Ala
145					150				155					160	
Gln	Tyr	Leu	Glu	Glu	Glu	Phe	Ile	Glu	Asp	His	Ala	Glu	Lys	Ile	Arg
			165					170					175		
Thr	Leu	Ala	Gly	His	Thr	Ser	Asp	Leu	Lys	Lys	Phe	Ile	Thr	Ala	Asn
			180					185					190		
Asn	Gly	His	Asp	Leu	Ser	Leu	Ala	Leu	Tyr	Val	Phe	Asp	Glu	Tyr	Leu
		195					200					205			
Gln	Lys	Thr	Val												
		210													

<210> 213

<211> 174

<212> PRT

<213> 激烈火球菌(*Pyrococcus furiosus*)

<400> 213

Met	Leu	Ser	Glu	Arg	Met	Leu	Lys	Ala	Leu	Asn	Asp	Gln	Leu	Asn	Arg
1				5					10					15	
Glu	Leu	Tyr	Ser	Ala	Tyr	Leu	Tyr	Phe	Ala	Met	Ala	Ala	Tyr	Phe	Glu
			20					25					30		

Asp	Leu	Gly	Leu	Glu	Gly	Phe	Ala	Asn	Trp	Met	Lys	Ala	Gln	Ala	Glu
35							40					45			
Glu	Glu	Ile	Gly	His	Ala	Leu	Arg	Phe	Tyr	Asn	Tyr	Ile	Tyr	Asp	Arg
50						55					60				
Asn	Gly	Arg	Val	Glu	Leu	Asp	Glu	Ile	Pro	Lys	Pro	Pro	Lys	Glu	Trp
65					70					75				80	
Glu	Ser	Pro	Leu	Lys	Ala	Phe	Glu	Ala	Ala	Tyr	Glu	His	Glu	Lys	Phe
				85					90					95	
Ile	Ser	Lys	Ser	Ile	Tyr	Glu	Leu	Ala	Ala	Leu	Ala	Glu	Glu	Glu	Lys
				100				105						110	
Asp	Tyr	Ser	Thr	Arg	Ala	Phe	Leu	Glu	Trp	Phe	Ile	Asn	Glu	Gln	Val
				115				120						125	
Glu	Glu	Glu	Ala	Ser	Val	Lys	Lys	Ile	Leu	Asp	Lys	Leu	Lys	Phe	Ala
						135					140				
Lys	Asp	Ser	Pro	Gln	Ile	Leu	Phe	Met	Leu	Asp	Lys	Glu	Leu	Ser	Ala
145					150					155					160
Arg	Ala	Pro	Lys	Leu	Pro	Gly	Leu	Leu	Met	Gln	Gly	Gly	Glu		
				165						170					

<210> 214

<211> 183

<212> PRT

<213> 智人(Homo sapiens)

<400> 214

Met	Thr	Thr	Ala	Ser	Thr	Ser	Gln	Val	Arg	Gln	Asn	Tyr	His	Gln	Asp
1				5					10					15	
Ser	Glu	Ala	Ala	Ile	Asn	Arg	Gln	Ile	Asn	Leu	Glu	Leu	Tyr	Ala	Ser
			20					25					30		
Tyr	Val	Tyr	Leu	Ser	Met	Ser	Tyr	Tyr	Phe	Asp	Arg	Asp	Asp	Val	Ala
		35					40					45			
Leu	Lys	Asn	Phe	Ala	Lys	Tyr	Phe	Leu	His	Gln	Ser	His	Glu	Glu	Arg
		50				55					60				
Glu	His	Ala	Glu	Lys	Leu	Met	Lys	Leu	Gln	Asn	Gln	Arg	Gly	Gly	Arg
65					70					75					80
Ile	Phe	Leu	Gln	Asp	Ile	Lys	Lys	Pro	Asp	Cys	Asp	Asp	Trp	Glu	Ser
				85					90					95	
Gly	Leu	Asn	Ala	Met	Glu	Cys	Ala	Leu	His	Leu	Glu	Lys	Asn	Val	Gln
				100					105					110	
Gln	Ser	Leu	Leu	Glu	Leu	His	Lys	Leu	Ala	Thr	Asp	Lys	Asn	Asp	Pro
				115					120					125	

His Leu Cys Asp Phe Ile Glu Thr His Tyr Leu Asn Glu Gln Val Lys
 130 135 140
 Ala Ile Lys Glu Leu Gly Asp His Val Thr Asn Leu Arg Lys Met Gly
 145 150 155 160
 Ala Pro Glu Ser Gly Leu Ala Glu Tyr Leu Phe Asp Lys His Thr Leu
 165 170 175
 Gly Asp Ser Asp Gln Glu Ser
 180
 <210> 215
 <211> 204
 <212> PRT
 <213> 智人(Homo sapiens)
 <400> 215
 Met Asp Ser Lys Gly Ser Ser Gln Lys Gly Ser Arg Leu Leu Leu Leu
 1 5 10 15
 Leu Val Val Ser Asn Leu Leu Leu Pro Gln Gly Val Leu Ala Ser Ser
 20 25 30
 Gln Ile Arg Gln Asn Tyr Ser Thr Asp Val Glu Ala Ala Val Asn Ser
 35 40 45
 Leu Val Asn Leu Tyr Leu Gln Ala Ser Tyr Thr Tyr Leu Ser Leu Gly
 50 55 60
 Phe Tyr Phe Asp Arg Asp Asp Val Ala Leu Glu Gly Val Ser His Phe
 65 70 75 80
 Phe Arg Glu Leu Ala Glu Glu Lys Arg Glu Gly Tyr Glu Arg Leu Leu
 85 90 95
 Lys Met Gln Asn Gln Arg Gly Gly Arg Ala Leu Phe Gln Asp Ile Lys
 100 105 110
 Lys Pro Ala Glu Asp Glu Trp Gly Lys Thr Pro Asp Ala Met Lys Ala
 115 120 125
 Ala Met Ala Leu Glu Lys Lys Leu Asn Gln Ala Leu Leu Asp Leu His
 130 135 140
 Ala Leu Gly Ser Ala Arg Thr Asp Pro His Leu Cys Asp Phe Leu Glu
 145 150 155 160
 Thr His Phe Leu Asp Glu Glu Val Lys Leu Ile Lys Lys Met Gly Asp
 165 170 175
 His Leu Thr Asn Leu His Arg Leu Gly Gly Pro Glu Ala Gly Leu Gly
 180 185 190
 Glu Tyr Leu Phe Glu Arg Leu Thr Leu Lys His Asp
 195 200

<210> 216

<400> 216

000

<210> 217

<211> 8

<212> PRT

<213> 未知

<220>

<221> 来源

<223> /注释="未知的描述:

牛蛙接头序列"

<400> 217

Glu Ser Gln Val Arg Gln Gln Phe

1 5

<210> 218

<211> 15

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述: 合成

肽"

<400> 218

Cys Leu Val Pro Arg Gly Ser Leu Glu His His His His His His

1 5 10 15

<210> 219

<400> 219

000

<210> 220

<211> 16

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述: 合成

肽"

<400> 220

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly

1 5 10 15

<210> 221

<211> 28

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成肽"

<400> 221

Gly	Gly	Ser	Gly	Ser	Gly	Ser	Asn	Ser	Ser	Ala	Ser	Ser	Gly	Ala	Ser
1				5				10					15		
Ser	Gly	Gly	Ala	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly				
			20					25							

<210> 222

<211> 46

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成多肽"

<400> 222

Gly	Gly	Ser	Gly	Ser	Ala	Ser	Ser	Gly	Ala	Ser	Ala	Ser	Gly	Ser	Ser
1				5				10					15		
Asn	Gly	Ser	Gly	Ser	Gly	Ser	Gly	Ser	Asn	Ser	Ser	Ala	Ser	Ser	Gly
			20					25				30			
Ala	Ser	Ser	Gly	Gly	Ala	Ser	Gly	Gly	Ser	Gly	Gly	Ser	Gly		
			35				40					45			

<210> 223

<211> 26

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成肽"

<400> 223

Gly	Gly	Ser	Gly	Ser	Ala	Ser	Ala	Glu	Ala	Ala	Ala	Lys	Glu	Ala	Ala
1				5				10					15		

Ala Lys Ala Gly Gly Ser Gly Gly Ser Gly
20 25

<210> 224

<211> 32

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成
多肽"

<400> 224

Gly Gly Ser Gly Ser Ala Ser Ala Glu Ala Ala Ala Lys Glu Ala Ala
1 5 10 15
Ala Lys Glu Ala Ala Ala Lys Ala Ser Gly Gly Ser Gly Gly Ser Gly
20 25 30

<210> 225

<211> 47

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成
多肽"

<400> 225

Ser Gly Gly Gly Ser Gly Ser Ala Ser Ser Gly Ala Ser Ala Ser Gly
1 5 10 15
Ser Ser Cys Ser Gly Ser Gly Ser Gly Ser Ser Ser Ala Ser Ser Gly
20 25 30
Ala Ser Ser Gly Gly Ala Ser Gly Gly Gly Ser Gly Gly Ser Gly
35 40 45

<210> 226

<211> 4

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成
肽"

<400> 226

Gly Gly Gly Ser

1

<210> 227

<211> 8

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成肽"

<400> 227

Gly Gly Gly Ser Gly Gly Gly Ser

1

5

<210> 228

<211> 20

<212> PRT

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成肽"

<400> 228

Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser

1

5

10

15

Gly Gly Gly Ser

20

<210> 229

<211> 5

<212> PRT

<213> 未知

<220>

<221> 来源

<223> /注释="未知的描述：单碱基突变序列"

<400> 229

Pro Gln Arg Glu Thr

1

5

<210> 230

<211> 20

<212> DNA

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成寡核苷酸"

<400> 230

tccatgacgt tcctgacgtt 20

<210> 231

<211> 21

<212> DNA

<213> 人工序列

<220>

<221> 来源

<223> /注释="人工序列的描述：合成寡核苷酸"

<400> 231

tgactgtgaa cgttcgagat g 21

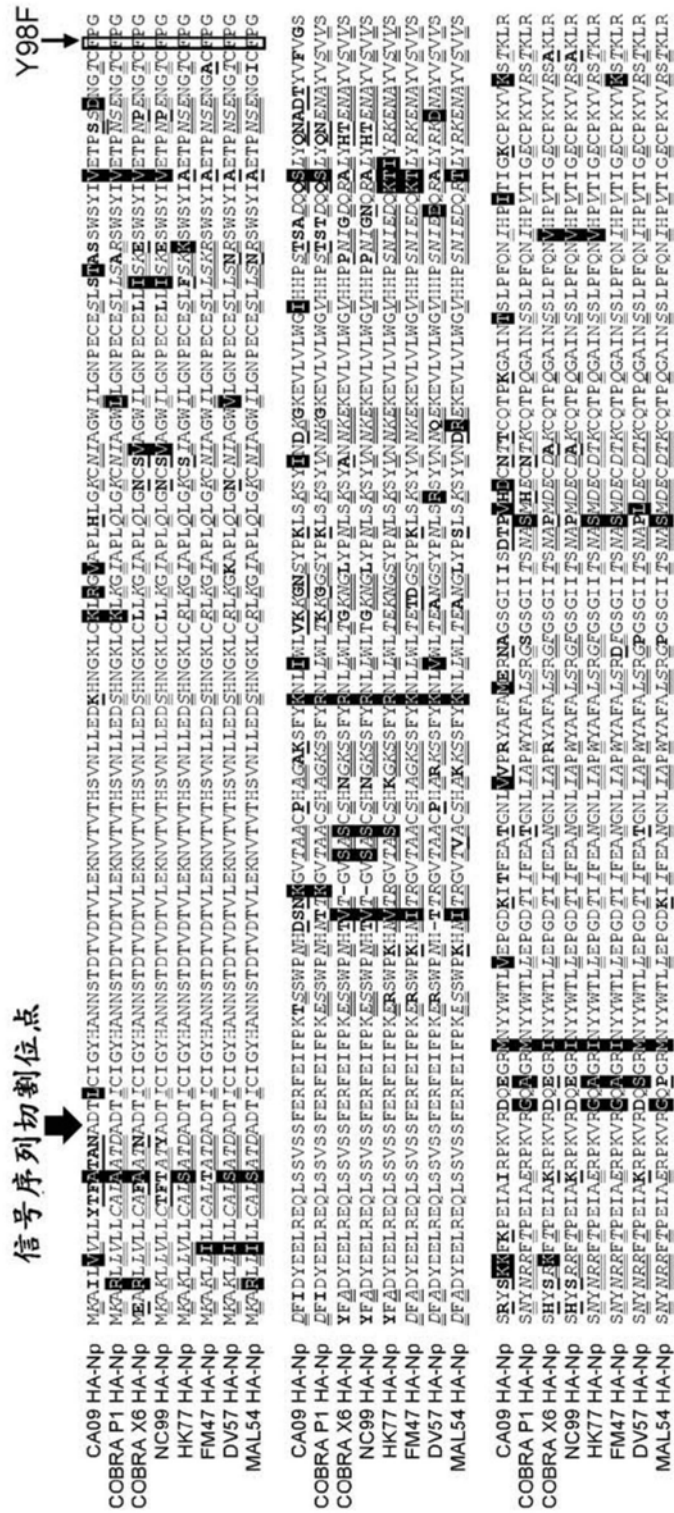


图1A

CA09 HA-Np	<u>LA</u> TGLRNI <u>PS</u> IOSRGLFGAIAGFIEGGWTG <u>MD</u> GWYGYHHQNEQSGYAAD <u>LK</u> STQNAI <u>DE</u> ITNKVNSVIEKMNTOFTAVGKEFN <u>HLE</u> AF <u>I</u> ENLNKKVDDGFLDIWTYNA
COBRA P1 HA-Np	<u>MT</u> TGLRNI <u>PS</u> IOSRGLFGAIAGFIEGGWTG <u>MD</u> GWYGYHHQNEQSGYAAD <u>OK</u> STQNAI <u>NG</u> ITNKVNSVIEKMNTOFTAVGKEFN <u>LE</u> AF <u>ME</u> NLNKKVDDGFLDIWTYNA
COBRA X6 HA-Np	<u>MT</u> TGLRNI <u>PS</u> IOSRGLFGAIAGFIEGGWTG <u>MD</u> GWYGYHHQNEQSGYAAD <u>OK</u> STQNAI <u>NG</u> ITNKVNSVIEKMNTOFTAVGKEFN <u>LE</u> AF <u>ME</u> NLNKKVDDGFLDIWTYNA
NC99 HA-Np	<u>MT</u> TGLRNI <u>PORE</u> <u>PR</u> GLFGAIAGFIEGGWTG <u>MD</u> GWYGYHHQNEQSGYAAD <u>OK</u> STQNAI <u>NG</u> ITNKVNSVIEKMNTOFTAVGKEFN <u>LE</u> AF <u>ME</u> NLNKKVDDGFLDIWTYNA
HK77 HA-Np	<u>MT</u> TGLRNI <u>PS</u> IOSRGLFGAIAGFIEGGWTG <u>MD</u> GWYGYHHQNEQSGYAAD <u>OK</u> STQNAI <u>NG</u> ITNKVNSVIEKMNTOFTAVGKEFN <u>LE</u> AF <u>ME</u> NLNKKVDDGFLDIWTYNA
FM47 HA-Np	<u>MT</u> TGLRNI <u>PS</u> IOSRGLFGAIAGFIEGGWTG <u>MD</u> GWYGYHHQNEQSGYAAD <u>OK</u> STQNAI <u>NG</u> ITNKVNSVIEKMNTOFTAVGKEFN <u>LE</u> AF <u>ME</u> NLNKKVDDGFLDIWTYNA
DV57 HA-Np	<u>MT</u> TGLRNI <u>PS</u> IOSRGLFGAIAGFIEGGWTG <u>MD</u> GWYGYHHQNEQSGYAAD <u>OK</u> STQNAI <u>NG</u> ITNKVNSVIEKMNTOFTAVGKEFN <u>LE</u> AF <u>ME</u> NLNKKVDDGFLDIWTYNA
MAL54 HA-Np	<u>MT</u> TGLRNI <u>PS</u> IOSRGLFGAIAGFIEGGWTG <u>MD</u> GWYGYHHQNEQSGYAAD <u>OK</u> STQNAI <u>NG</u> ITNKVNSVIEKMNTOFTAVGKEFN <u>LE</u> AF <u>ME</u> NLNKKVDDGFLDIWTYNA
CA09 HA-Np	ELLVLLNERTLD <u>FD</u> HDSNVKNLYEKV <u>ASQI</u> ANNAAKE <u>IGN</u> GCFEYFYHKD <u>NT</u> CMESVKNGTVDYPKYSE <u>EL</u> KLNR <u>ED</u> IDS
COBRA P1 HA-Np	ELLVLLNERTLD <u>FD</u> HDSNVKNLYEKV <u>ASQI</u> ANNAAKE <u>IGN</u> GCFEYFYHKD <u>NT</u> CMESVKNGTVDYPKYSE <u>EL</u> KLNR <u>ED</u> IDS
COBRA X6 HA-Np	ELLVLLNERTLD <u>FD</u> HDSNVKNLYEKV <u>ASQI</u> ANNAAKE <u>IGN</u> GCFEYFYHKD <u>NT</u> CMESVKNGTVDYPKYSE <u>EL</u> KLNR <u>ED</u> IDS
NC99 HA-Np	ELLVLLNERTLD <u>FD</u> HDSNVKNLYEKV <u>ASQI</u> ANNAAKE <u>IGN</u> GCFEYFYHKD <u>NT</u> CMESVKNGTVDYPKYSE <u>EL</u> KLNR <u>ED</u> IDS
HK77 HA-Np	ELLVLLNERTLD <u>FD</u> HDSNVKNLYEKV <u>ASQI</u> ANNAAKE <u>IGN</u> GCFEYFYHKD <u>NT</u> CMESVKNGTVDYPKYSE <u>EL</u> KLNR <u>ED</u> IDS
FM47 HA-Np	ELLVLLNERTLD <u>FD</u> HDSNVKNLYEKV <u>ANQI</u> ANNAAKE <u>IGN</u> GCFEYFYHKD <u>NT</u> CMESVKNGTVDYPKYSE <u>EL</u> KLNR <u>ED</u> IDS
DV57 HA-Np	ELLVLLNERTLD <u>FD</u> HDSNVKNLYEKV <u>ANQI</u> ANNAAKE <u>IGN</u> GCFEYFYHKD <u>NT</u> CMESVKNGTVDYPKYSE <u>EL</u> KLNR <u>ED</u> IDS
MAL54 HA-Np	ELLVLLNERTLD <u>FD</u> HDSNVKNLYEKV <u>ANQI</u> ANNAAKE <u>IGN</u> GCFEYFYHKD <u>NT</u> CMESVKNGTVDYPKYSE <u>EL</u> KLNR <u>ED</u> IDS

图1A (续)

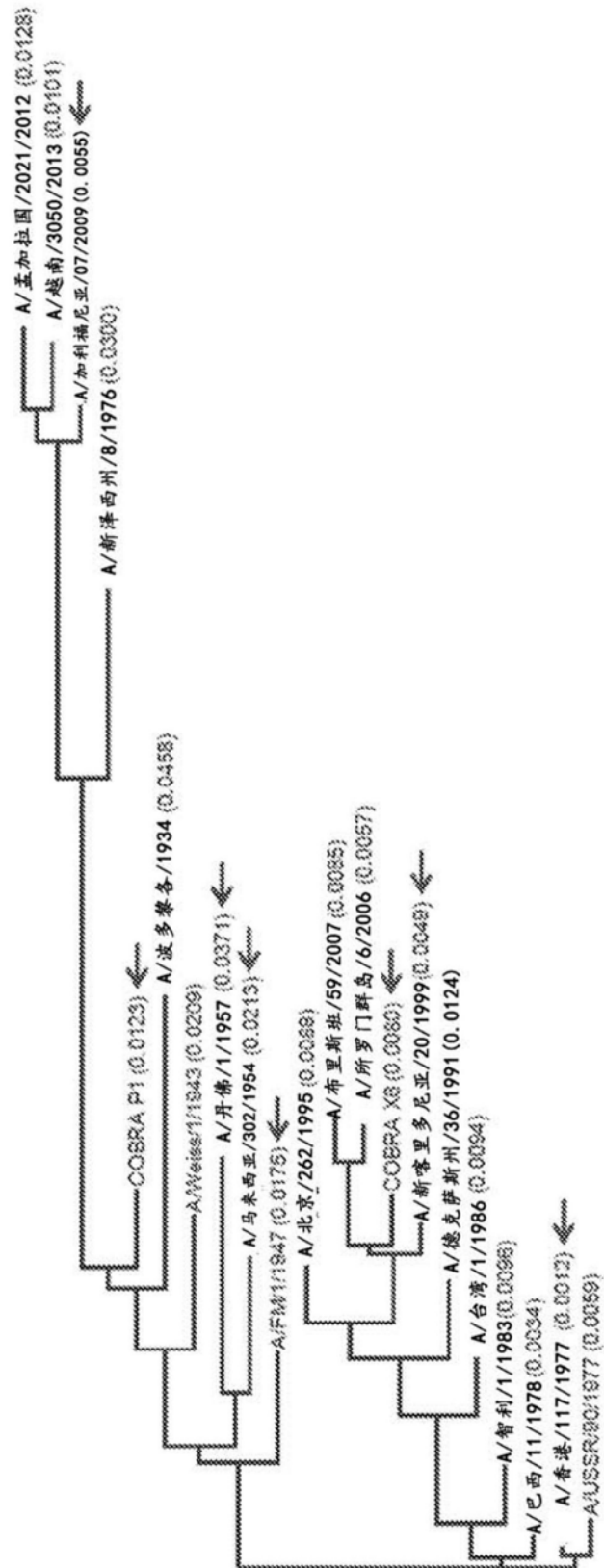


图1B

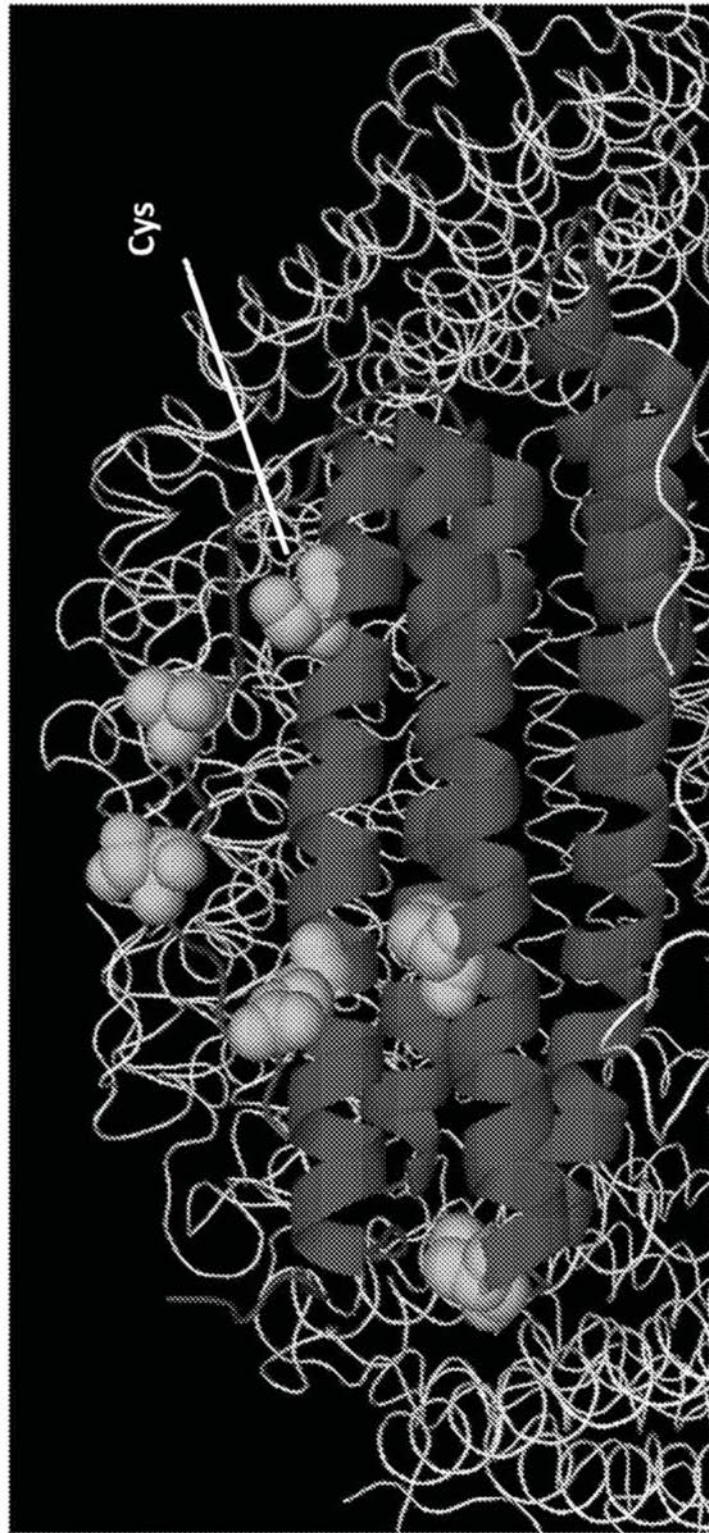


图2

1步式点击化学：在还原H1/茎-Np (N19Q、C31S、S111C) 后将SM7/8a-PEG4-马来酰亚胺亚胺位点特异性地缀合至不成对半胱氨酸 (Cys 111)。

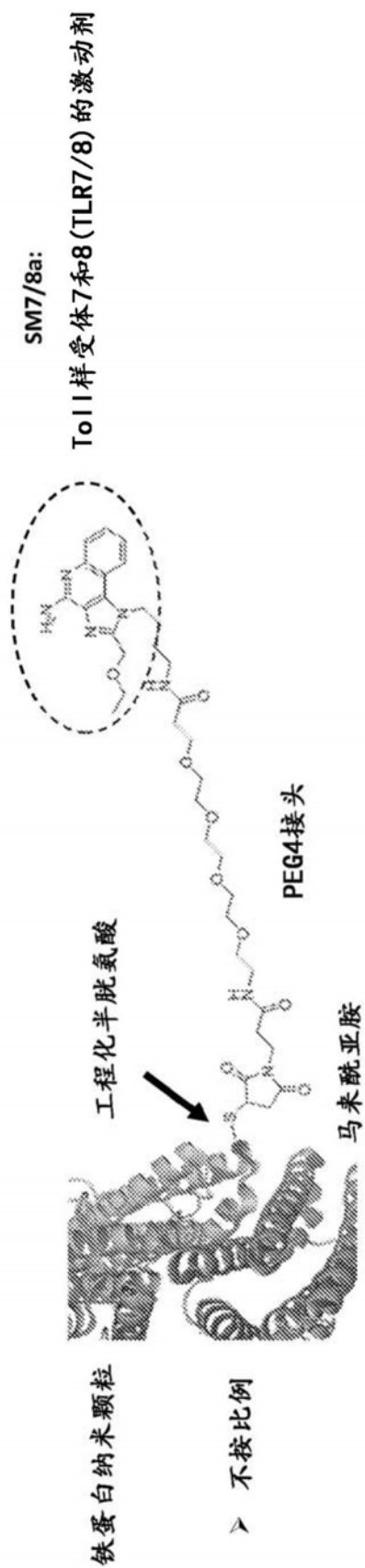


图3A

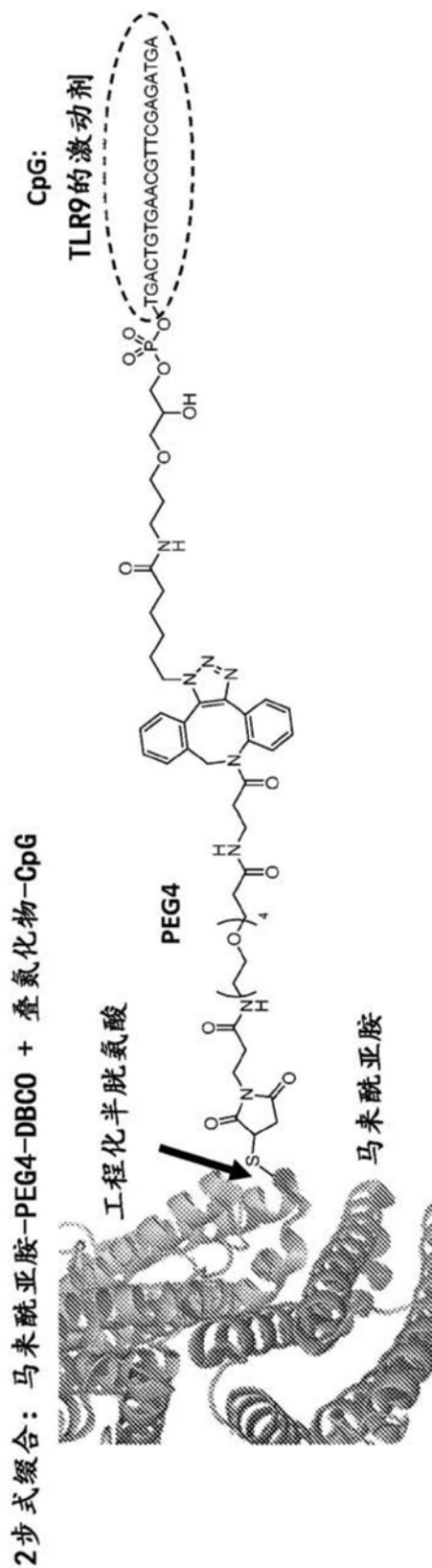


图3B

2步式点击化学：在将来酰亚胺-PEG4-DBC0接头与Cys 111缀合后，将3M012-叠氮化物添加至H1/茎-Np（N19Q、C31S、S111C）上的接头

HA-Ferr-3M012的模型

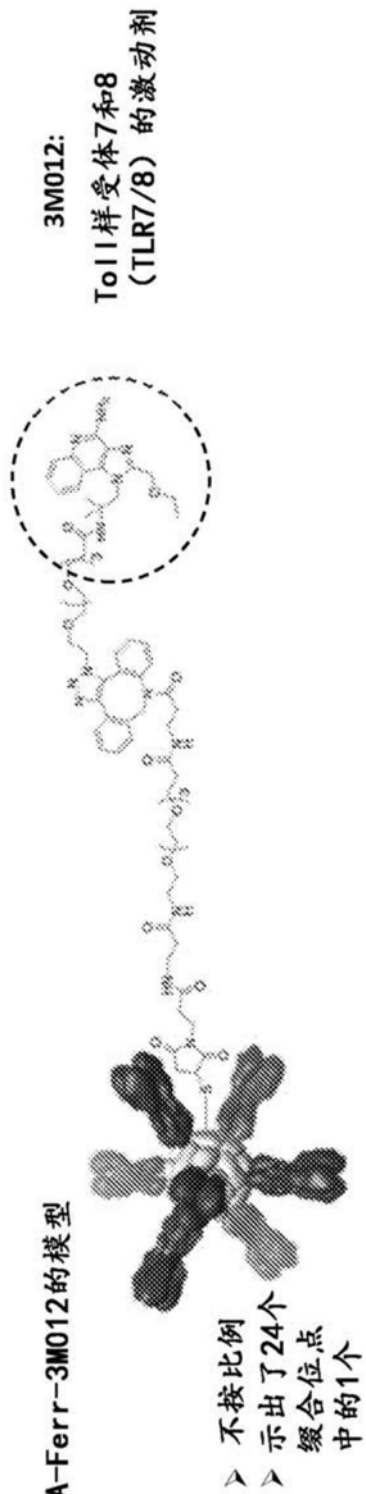


图4A

2步式点击化学：在将马来酰亚胺-PEG4-DBCO接头与Cys 111缀合后，
将CpG-叠氮化物添加至H1/茎-Np (N19Q、C31S、S111C) 上的接头

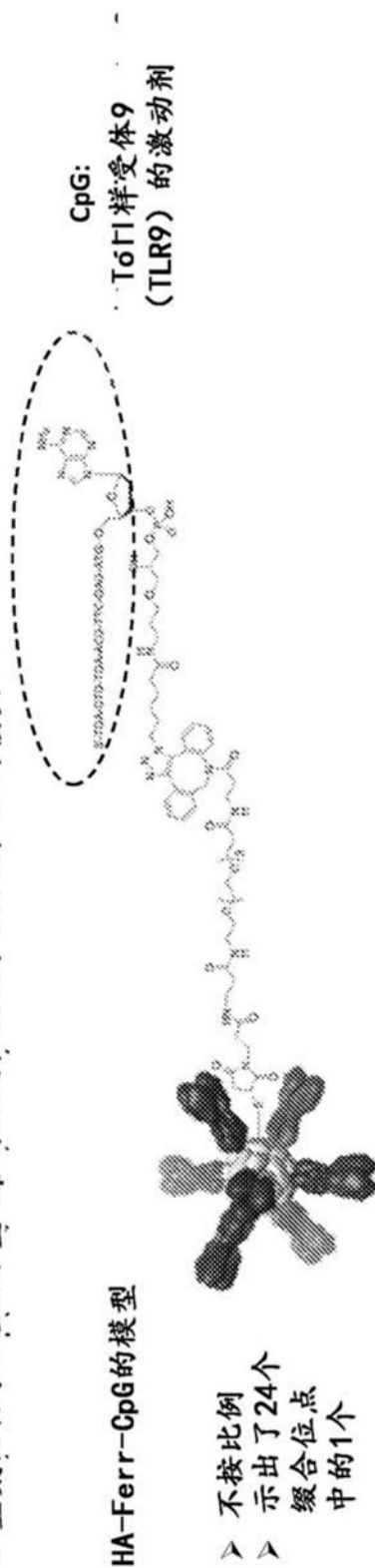


图4B

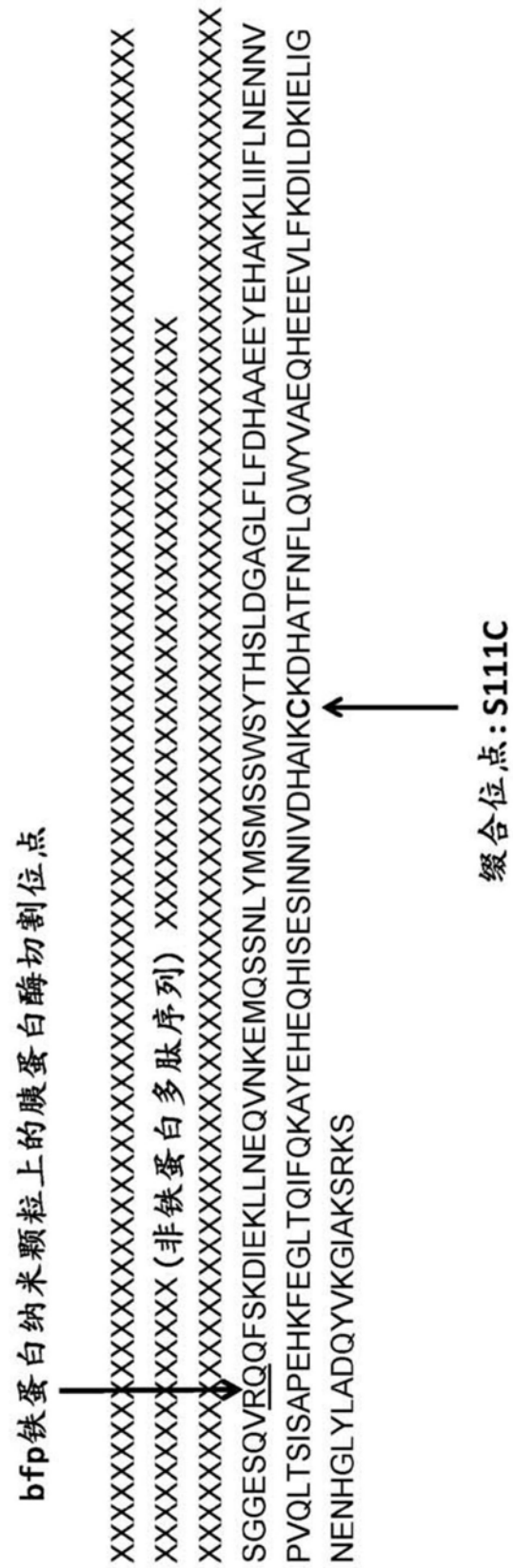


图5

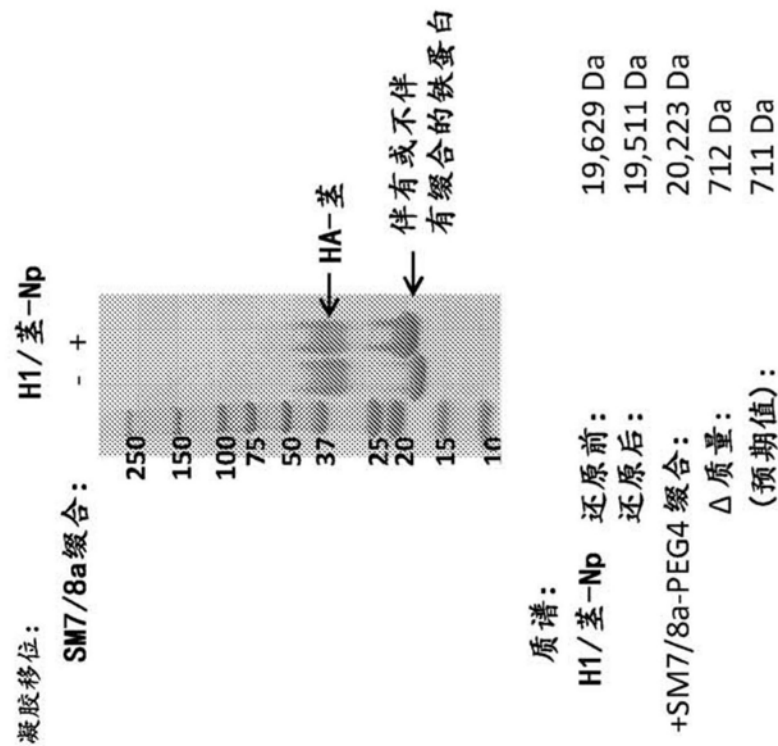


图6A

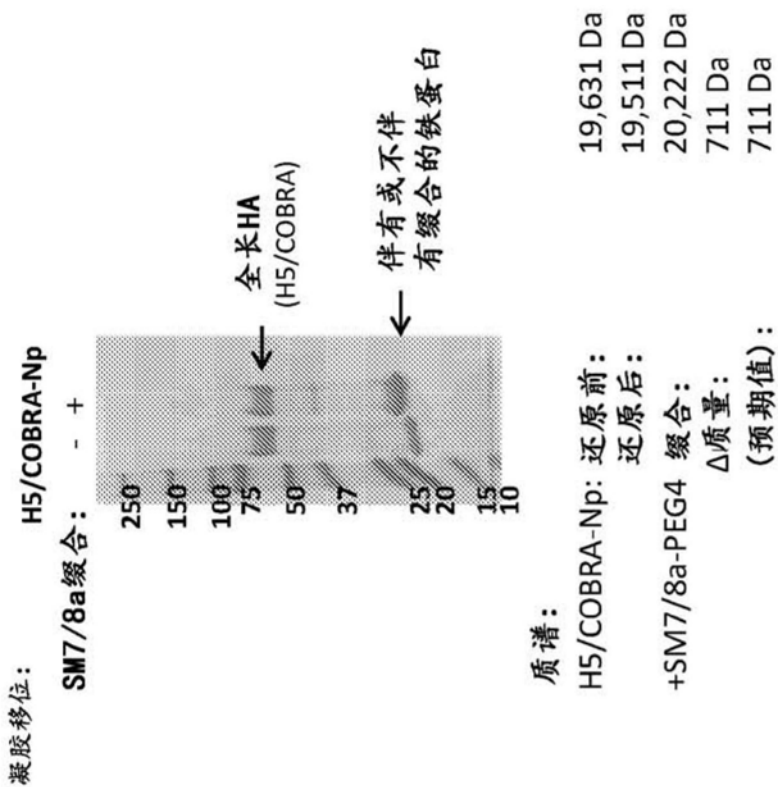


图6B

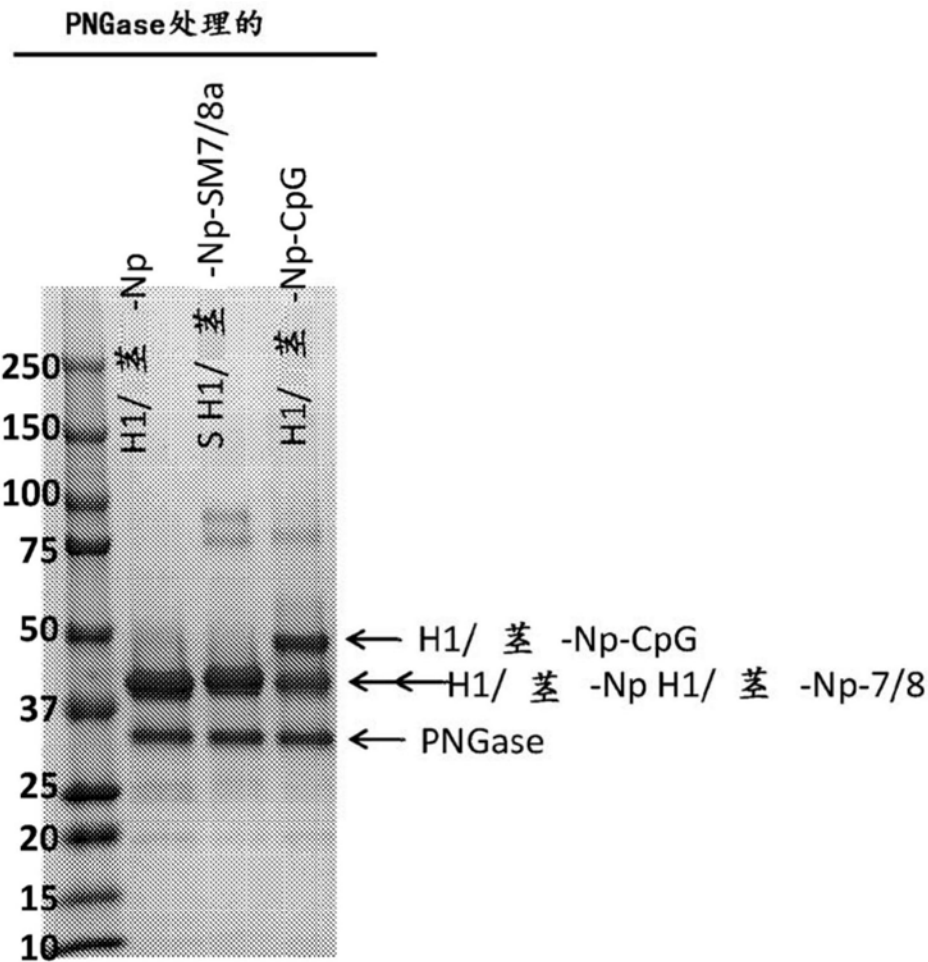


图7A

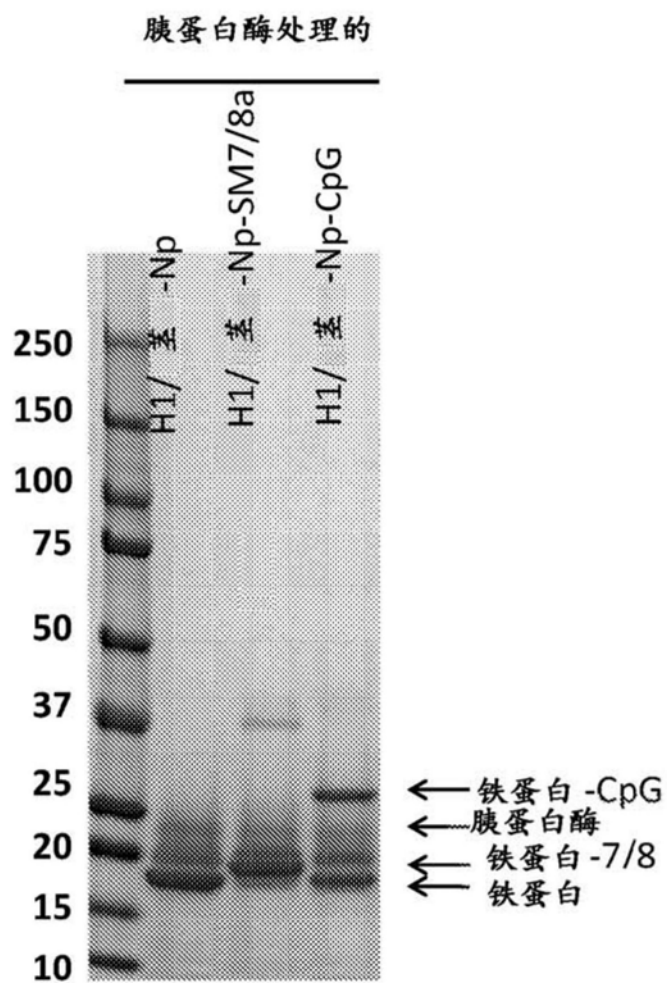


图7B

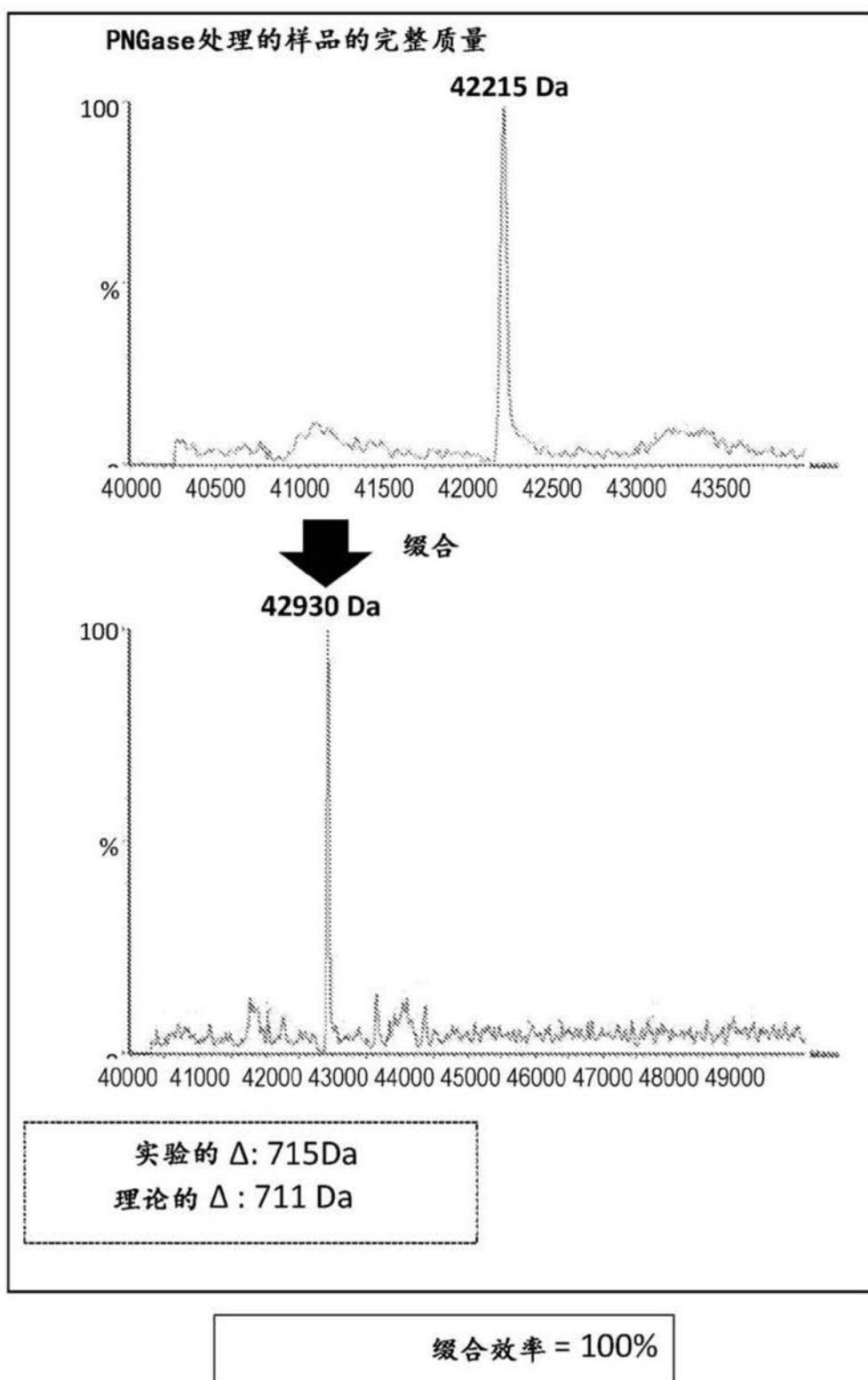


图8A

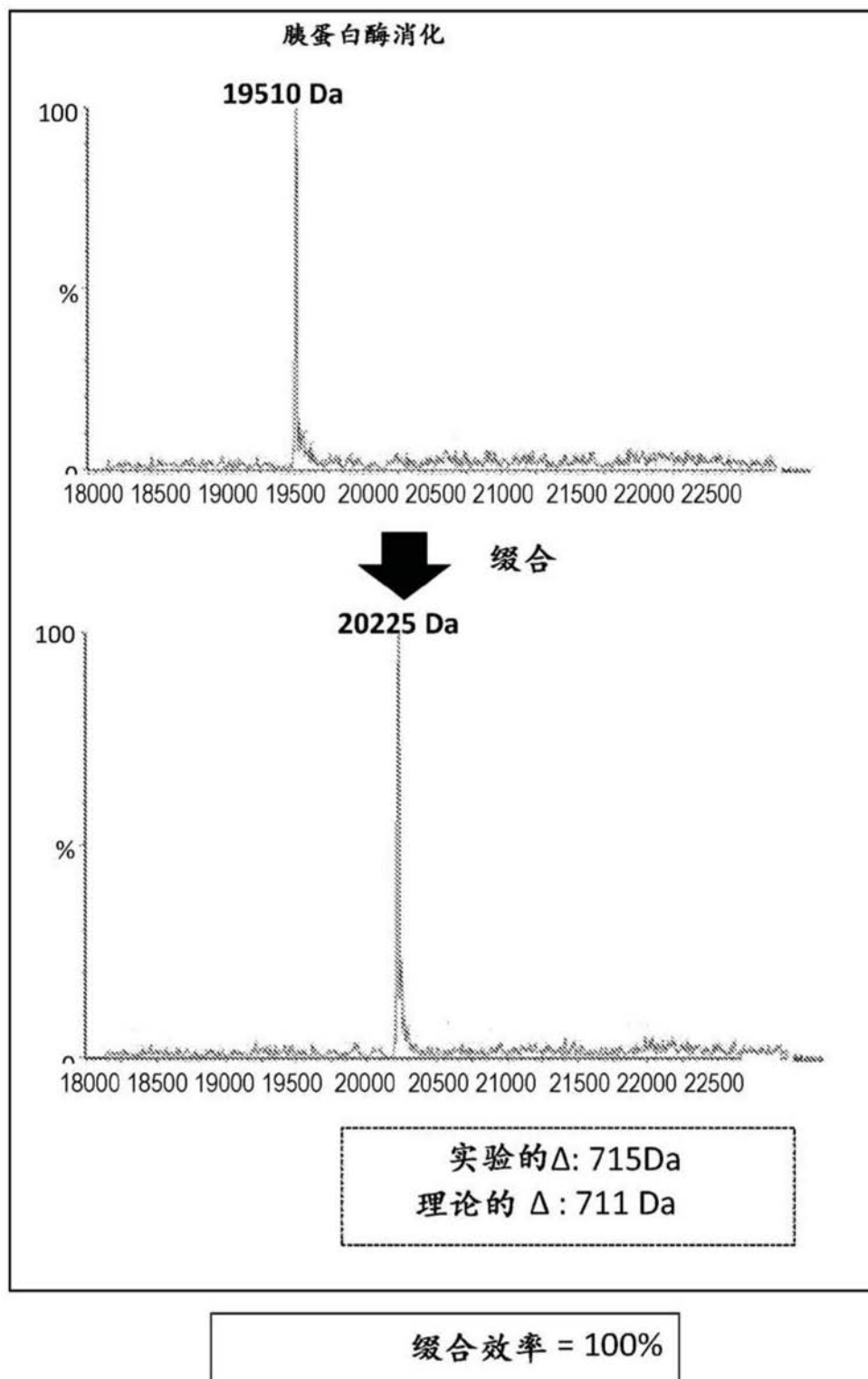


图8B

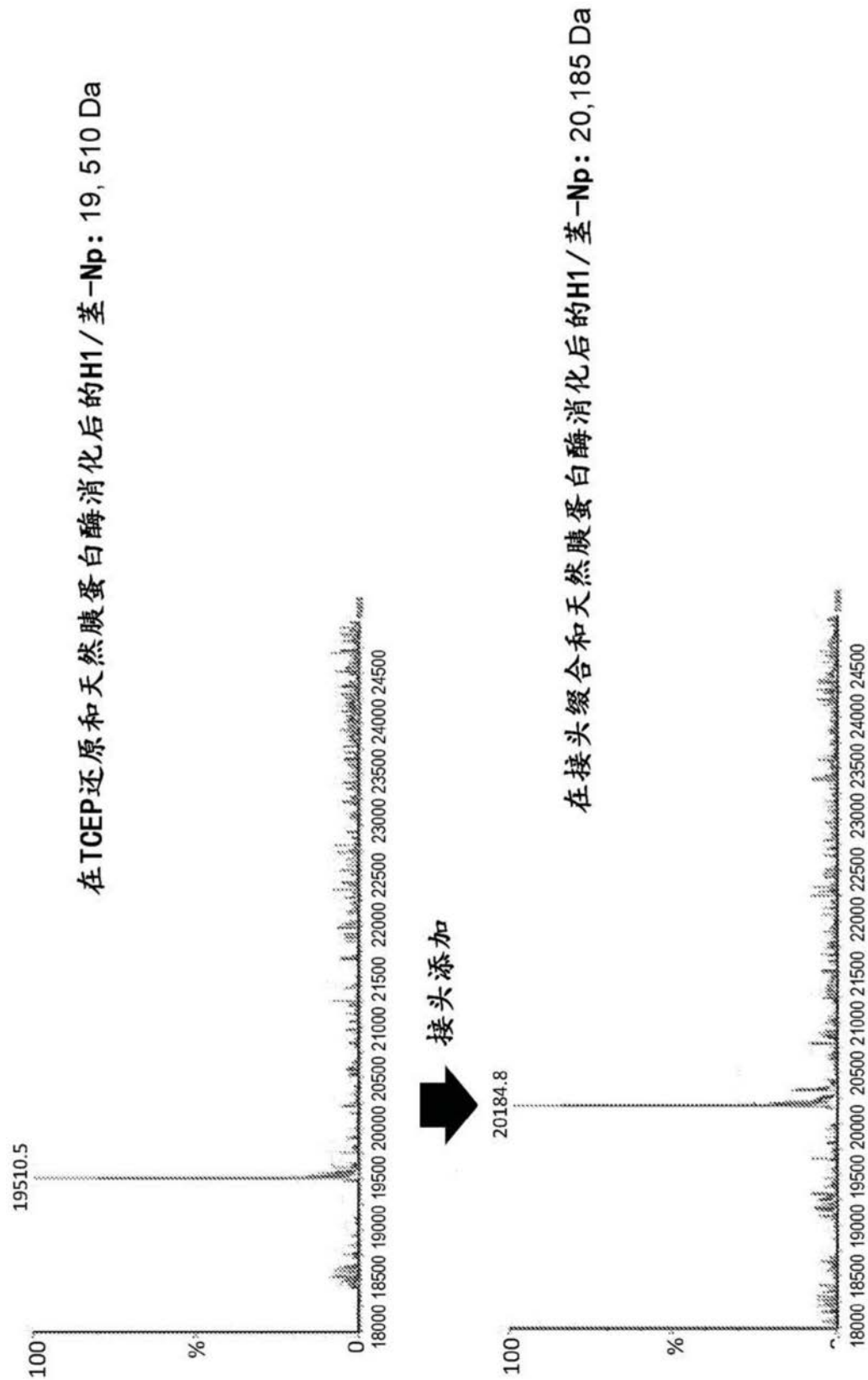


图9

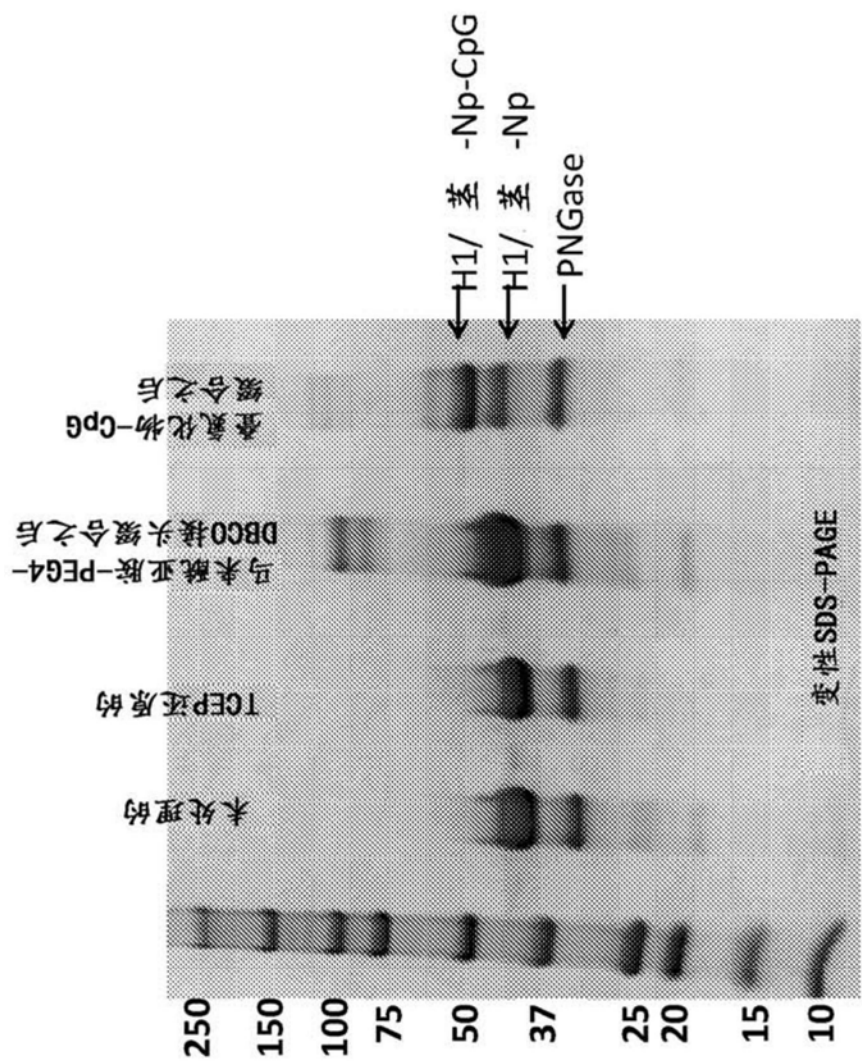


图10

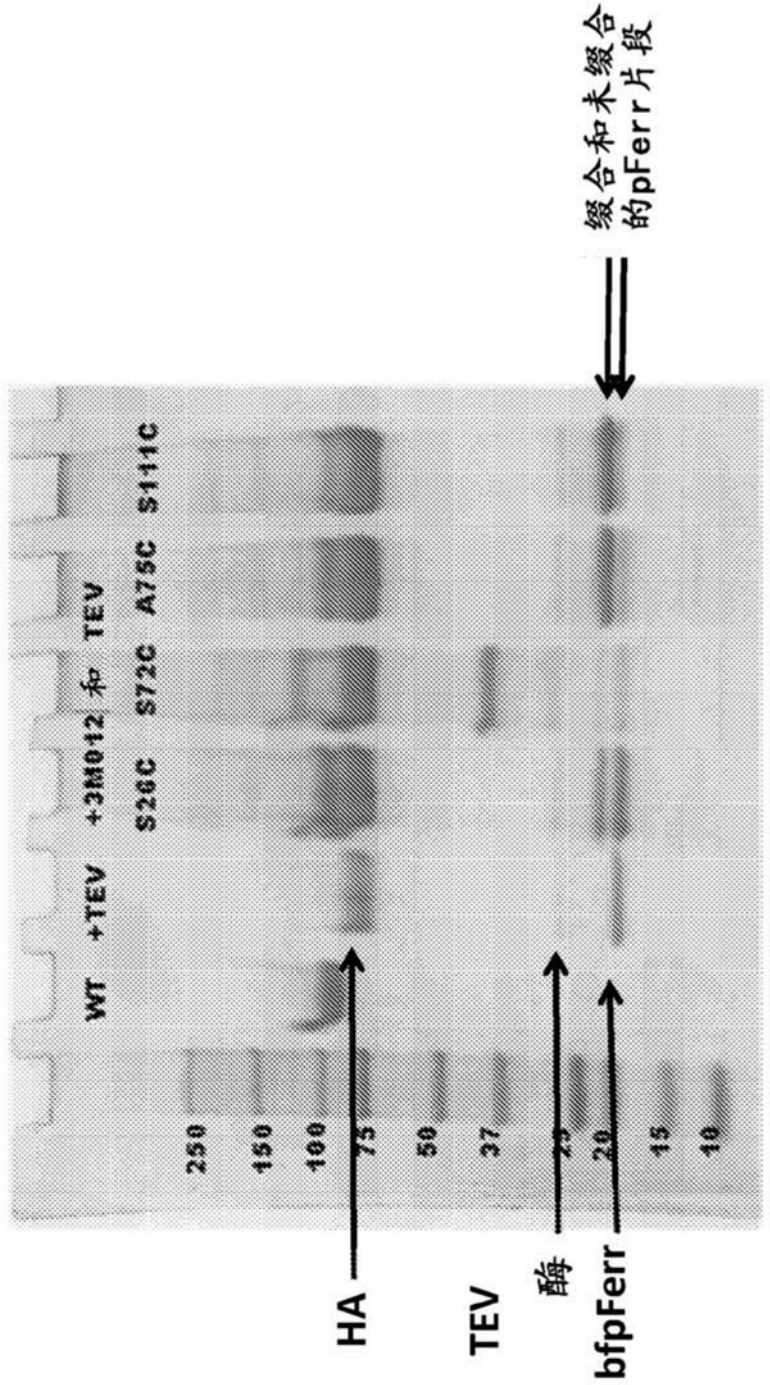


图11

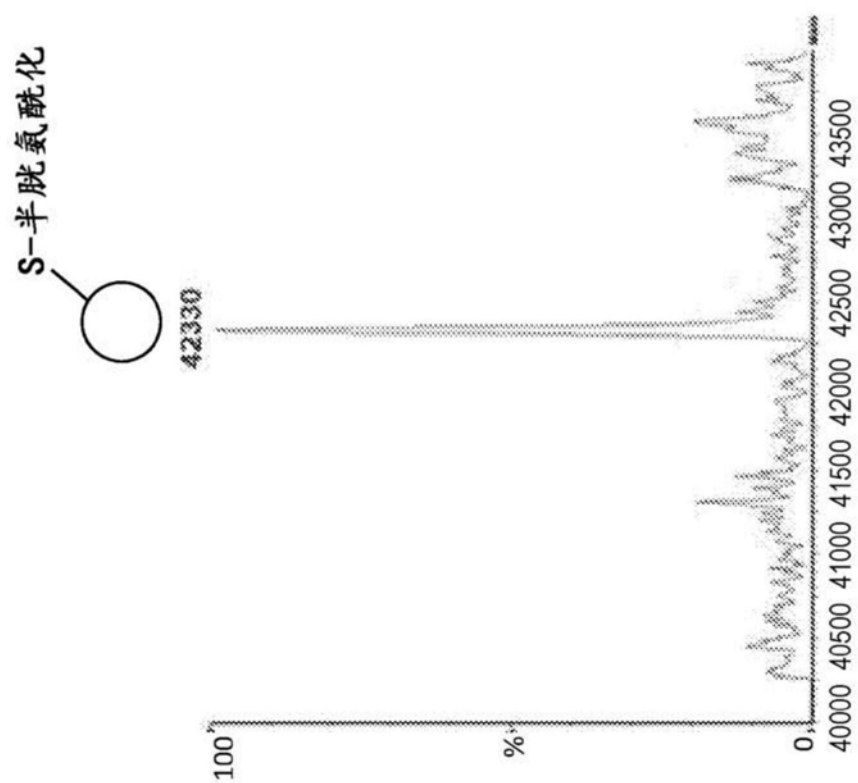


图12A

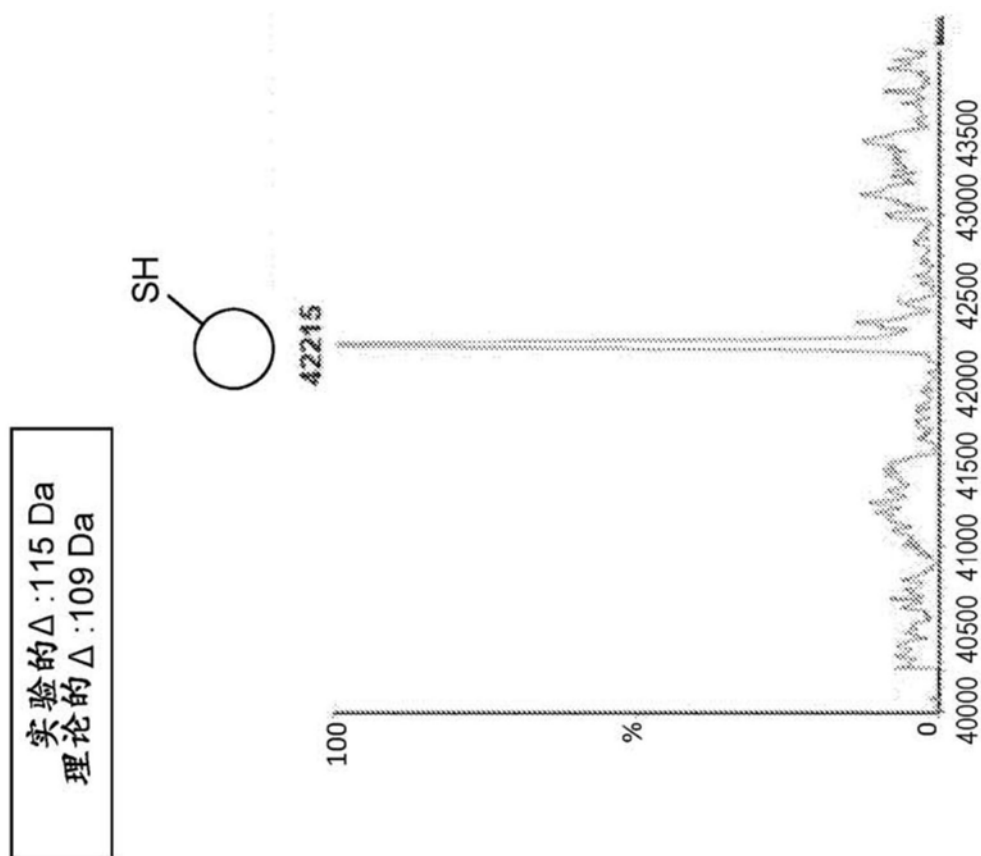


图12B

TEV切割的pFerr的完整质量分析

---SIB 6252: NC99 HA-TEV-Np-S26C

起始MW: 77397.69加上聚糖，切割后，pFerr MW: 20246.64

接头MW: 674.74 Da; 3M012-PEG4-叠氮化物: 586.69 Da

总质量移位1261.43 Da; 最终的缀合物: 21524.43 Da

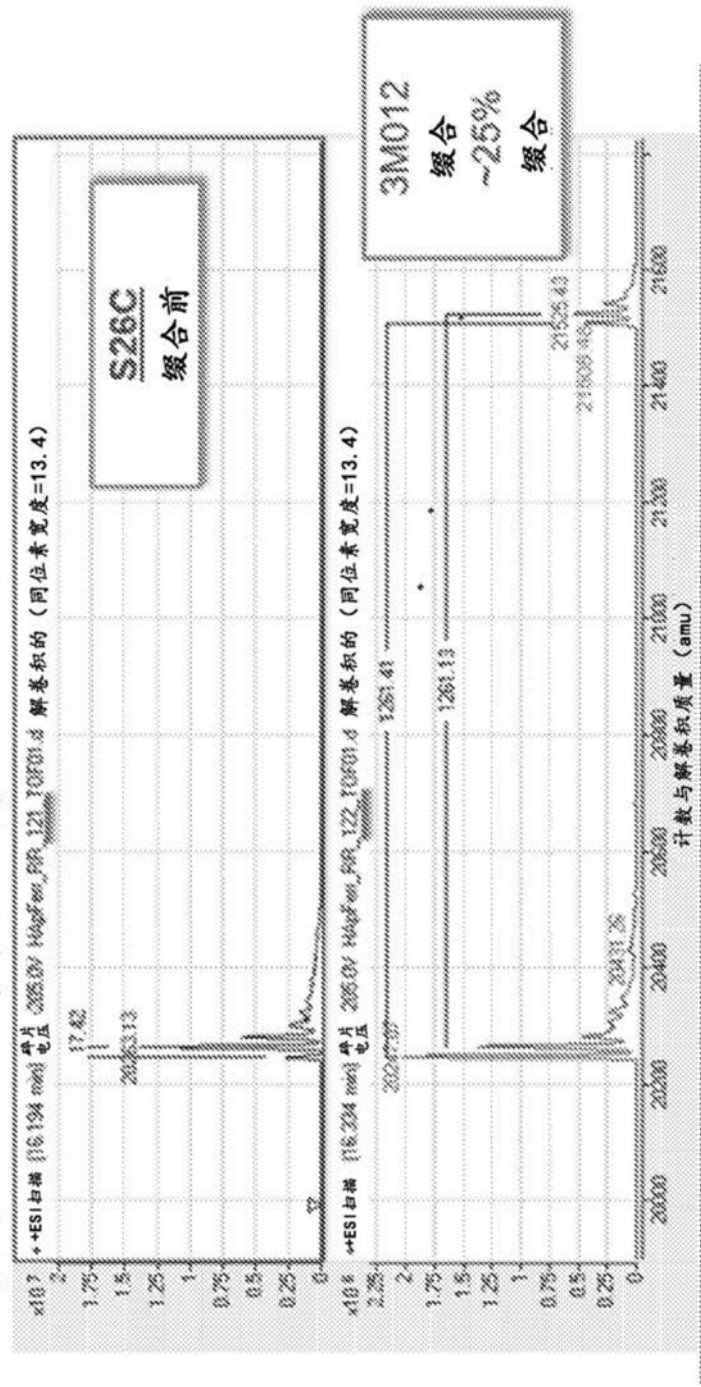


图12C

TEV切割的pFerr的完整质量分析

---SIB 6254: NC99 HA-TEV-Np-A75C

起始MW: 77413.69加上聚糖，切割后，pFerr MW: 20262.63
接头MW: 674.74 Da; 3M012-PEG4-叠氮化物: 586.69 Da
总质量移位1261.43 Da; 最终的缀合产物: 21524.43 Da

放大视图

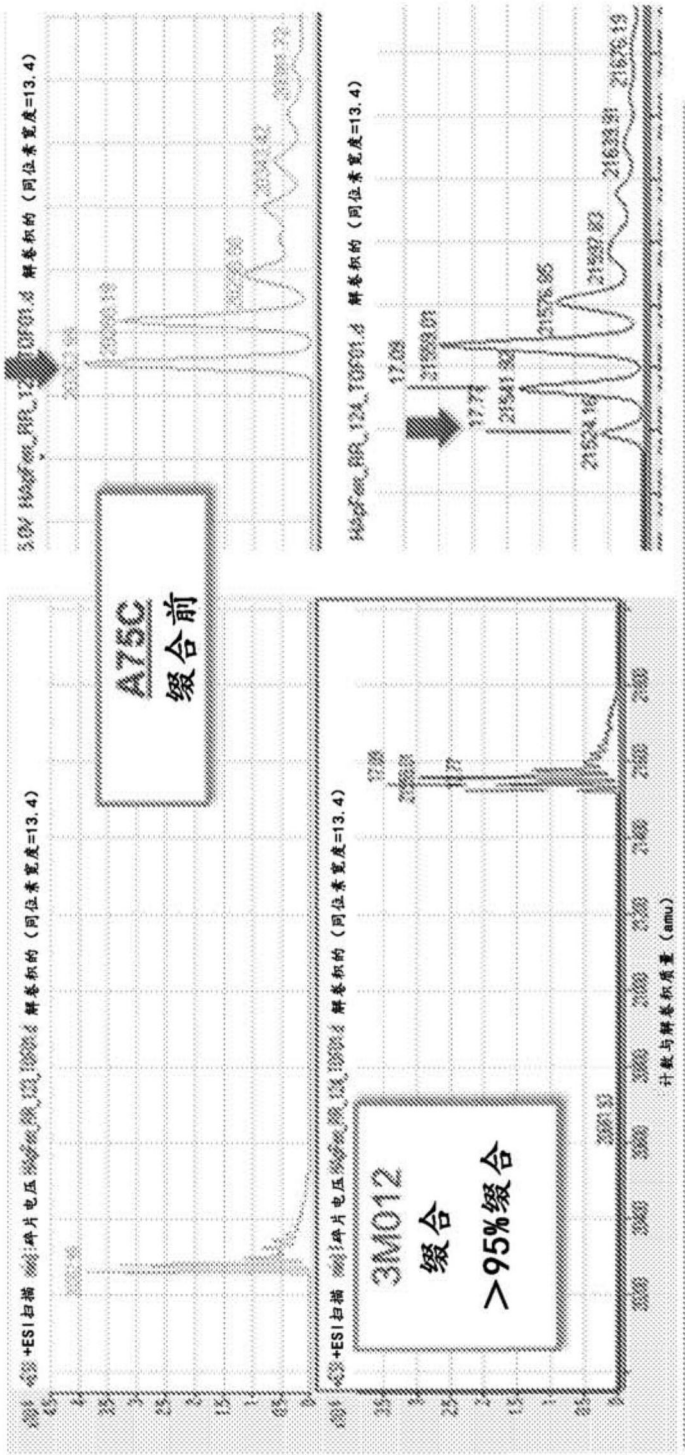


图12D

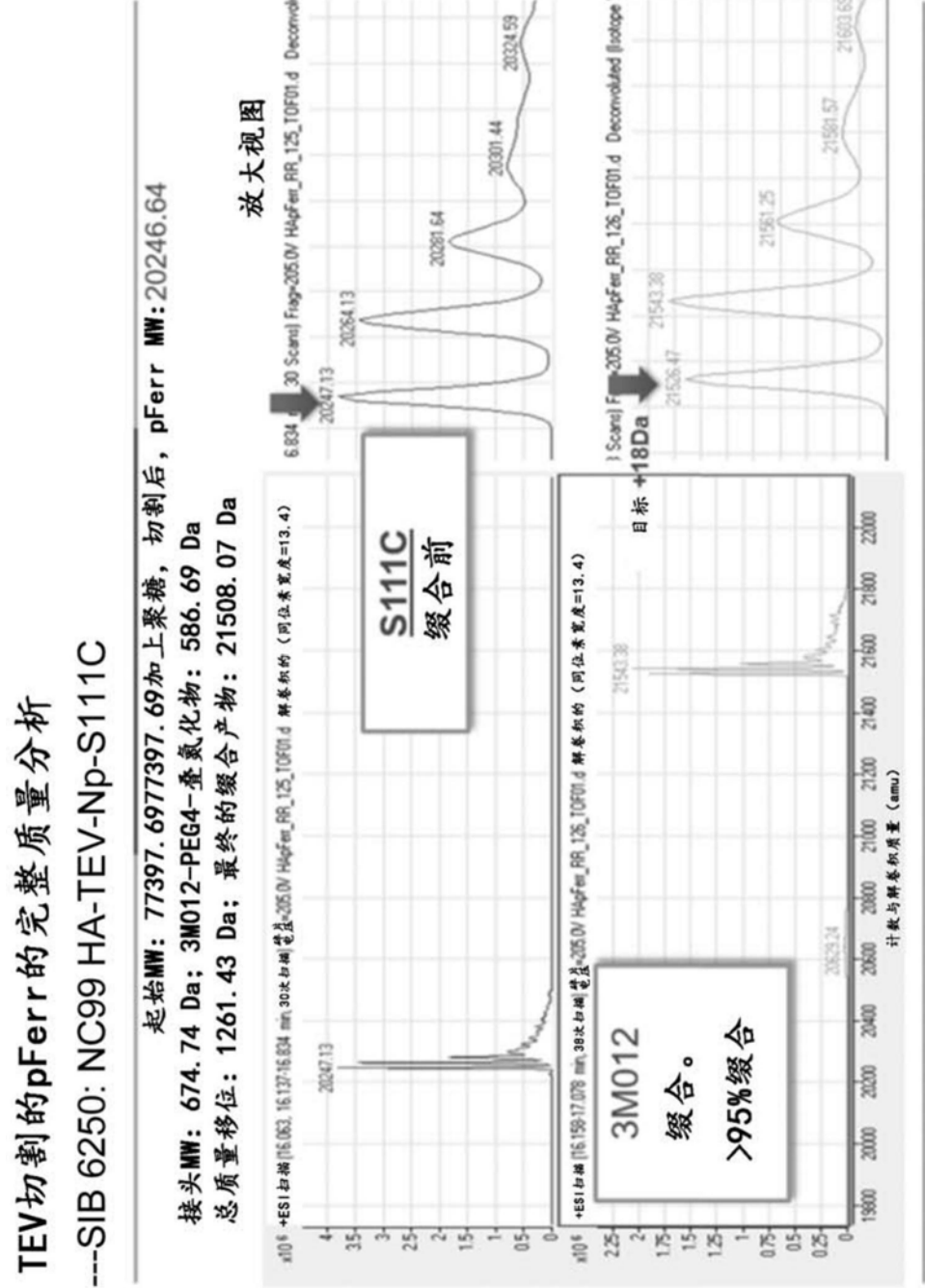


图12E

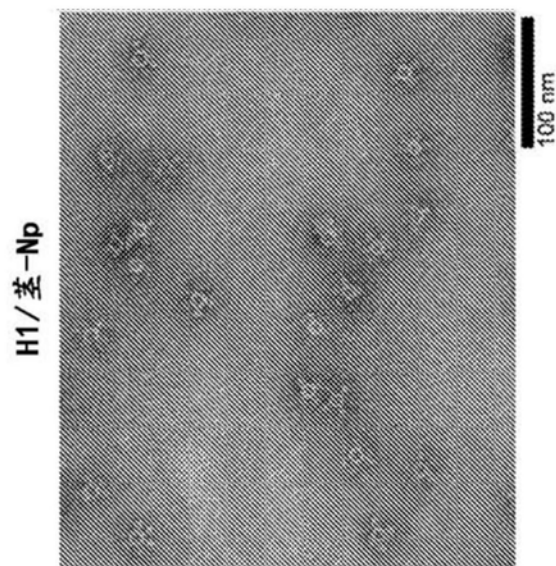


图13A

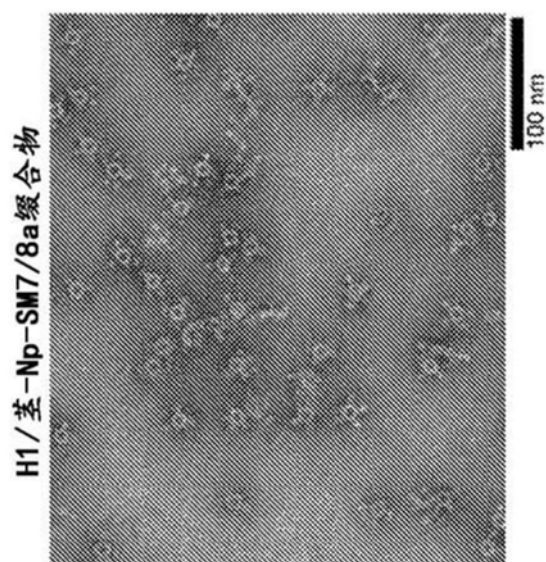


图13B

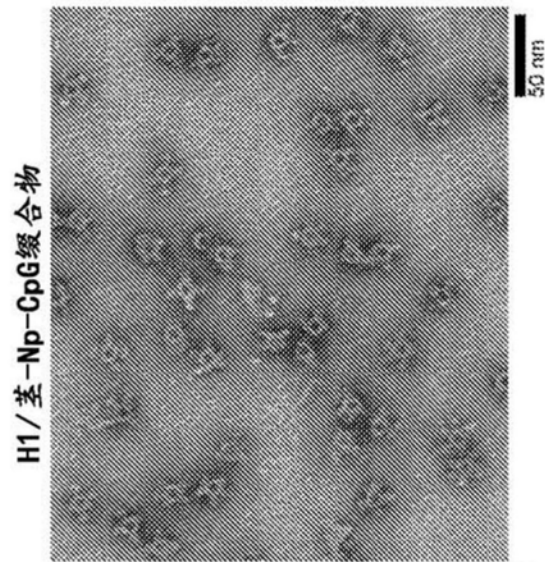


图13C

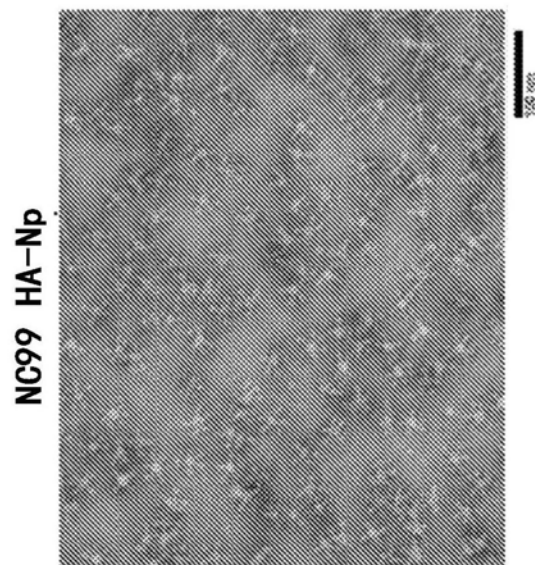


图13D

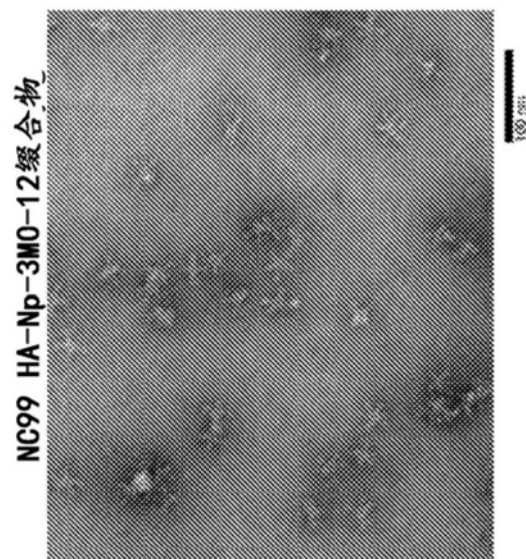


图13E

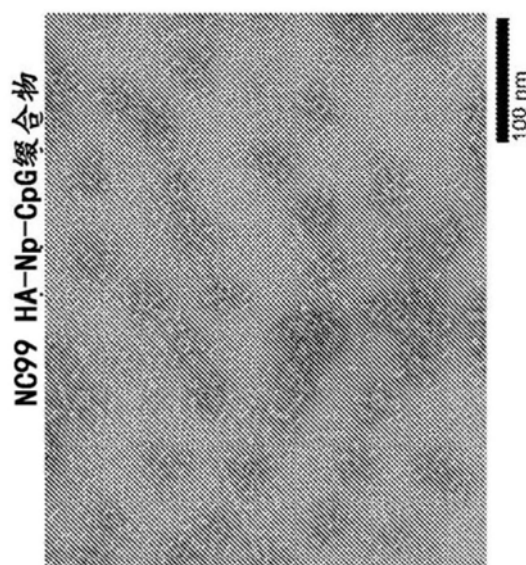


图13F

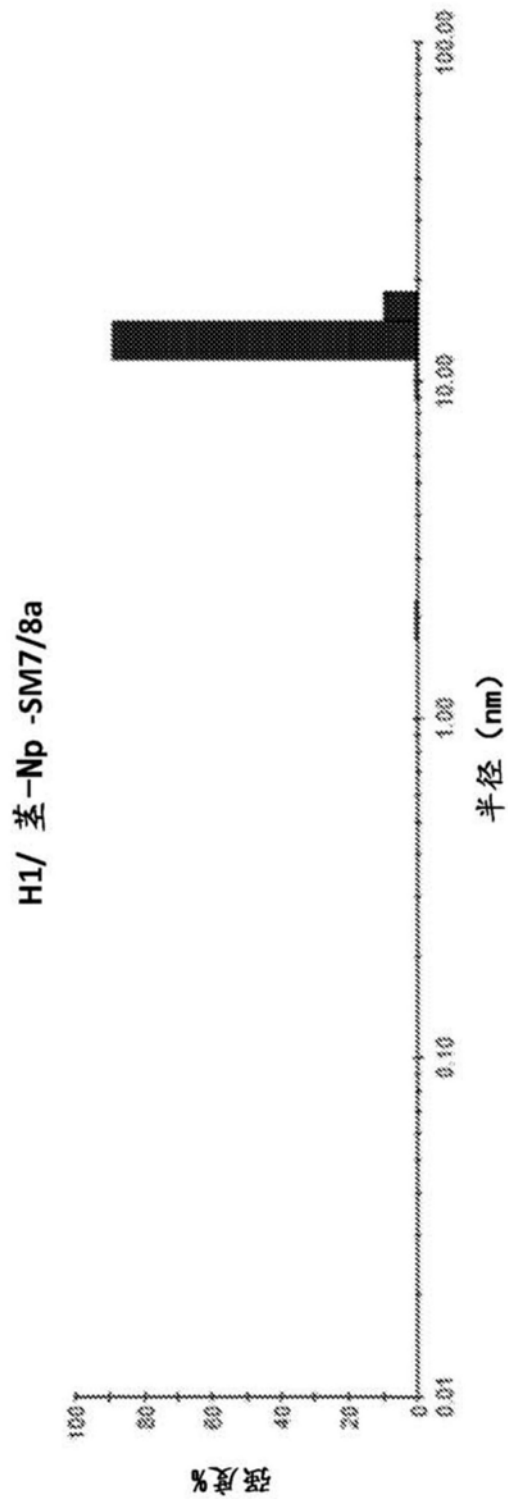


图14A

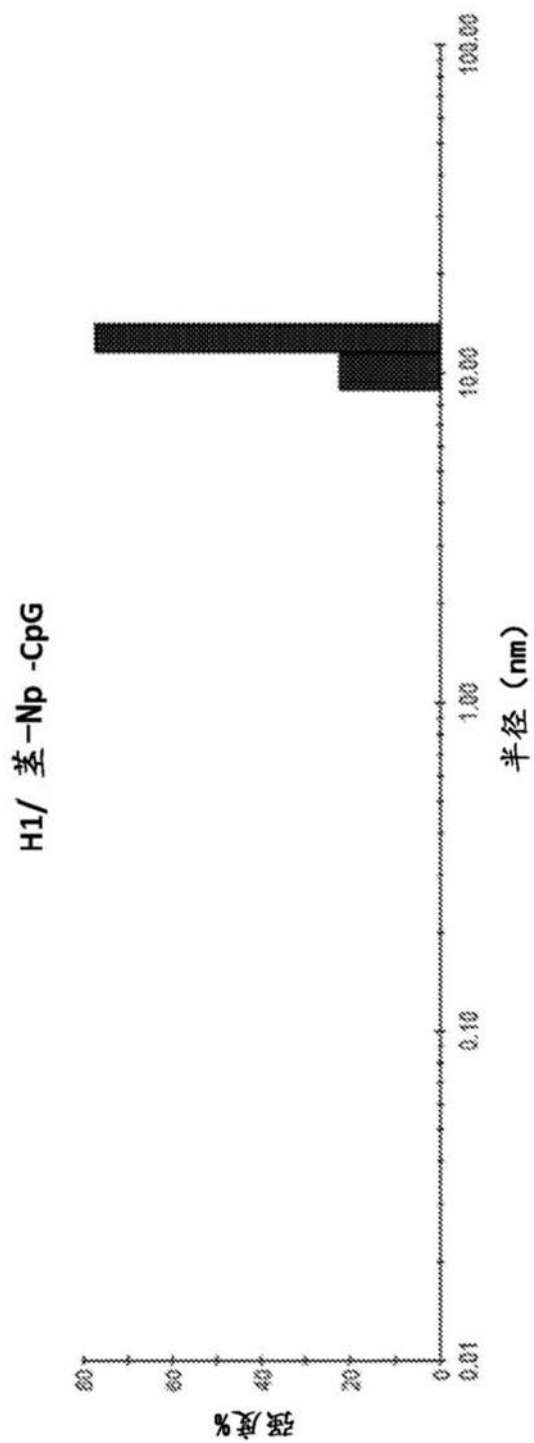


图14B

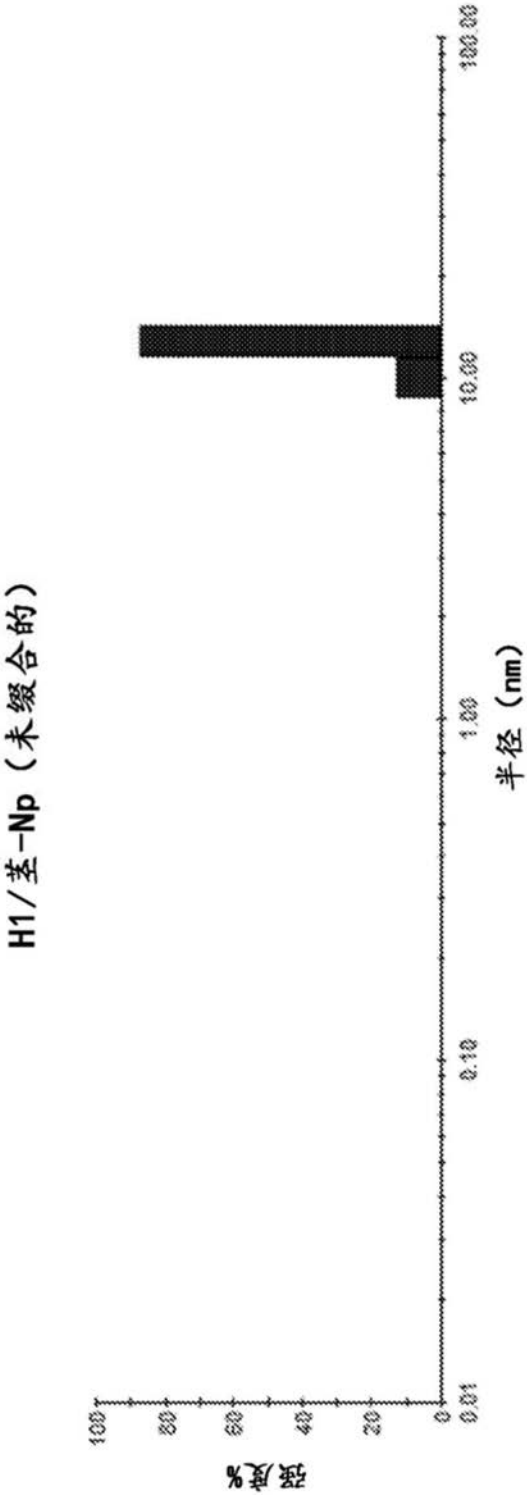
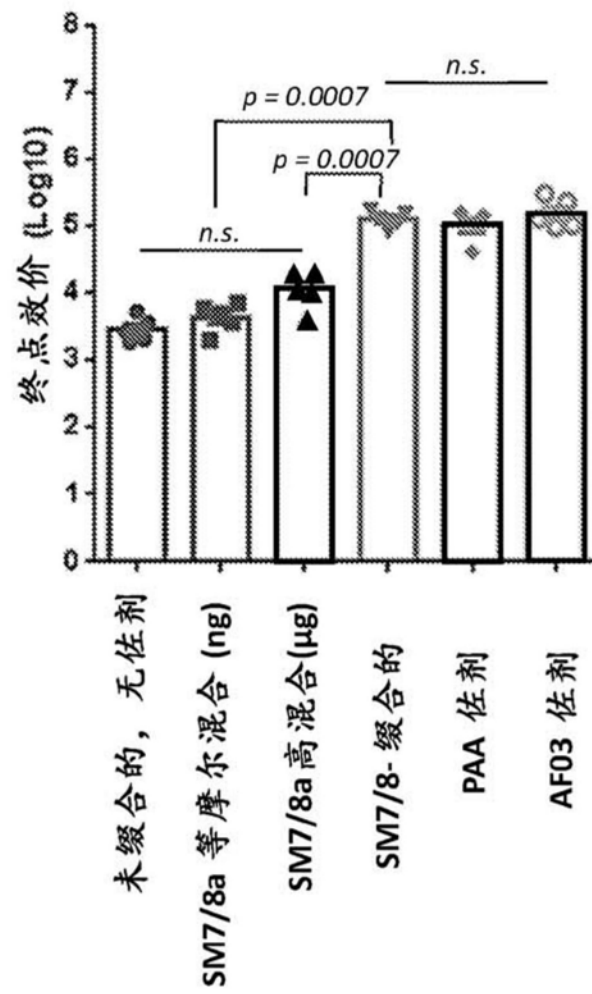


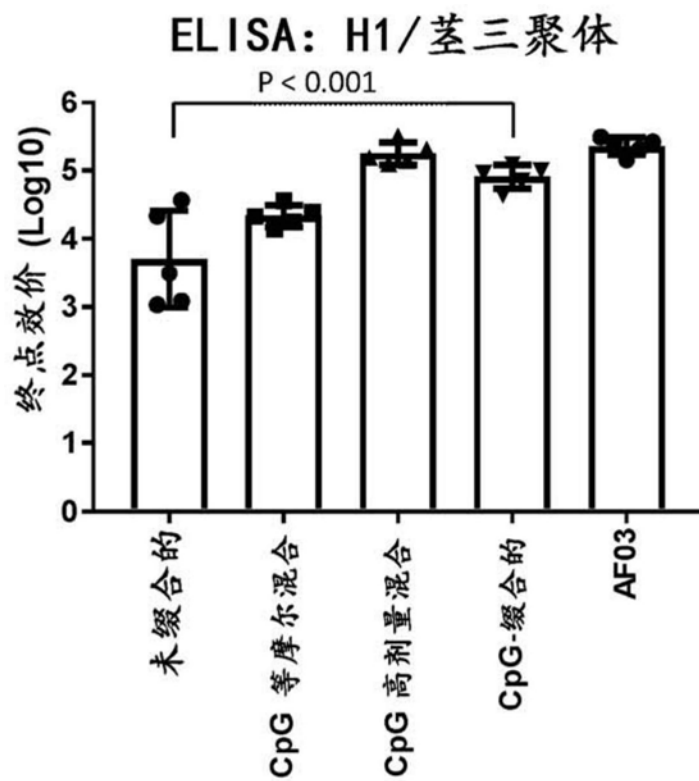
图14C

ELISA: H1/新喀里多尼亚/1999 HA三聚体



掺合的或缀合的H1/茎-Np抗原

图15A



掺合的或缀合的H1/茎-Np抗原

图15B

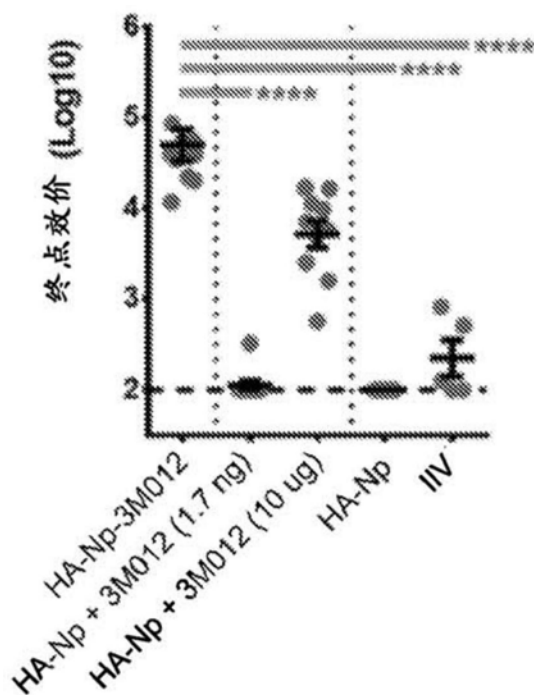


图16A

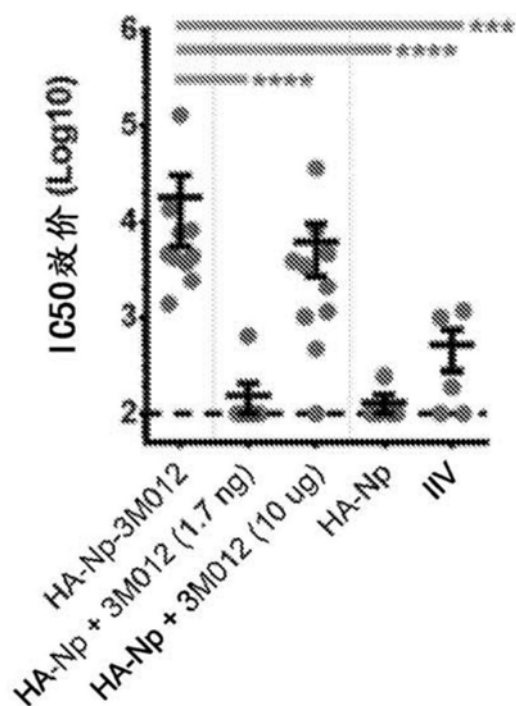


图16B

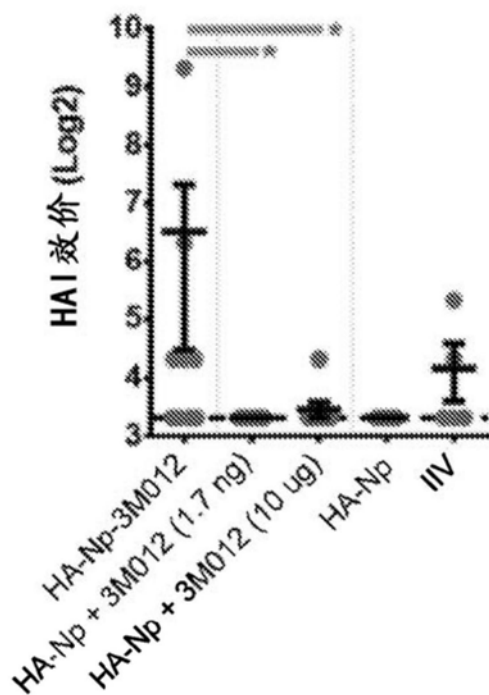


图16C

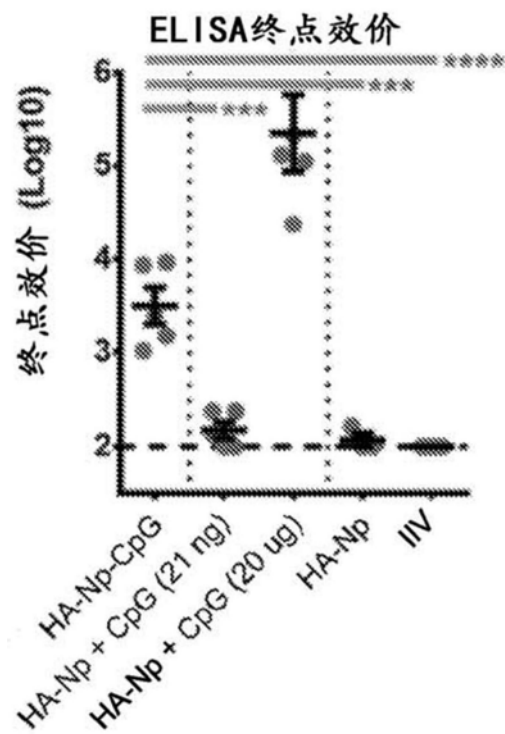


图17A

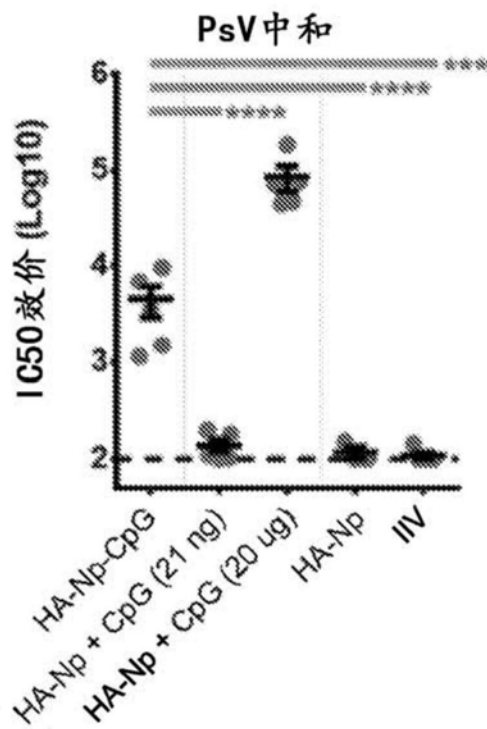


图17B

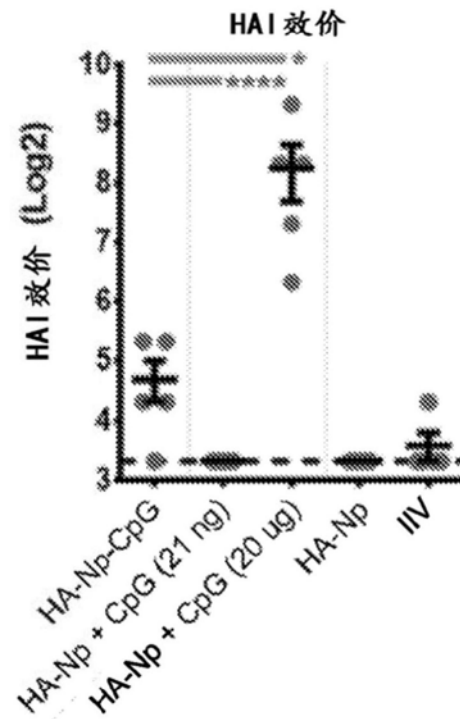


图17C

年份	纳米颗粒	大小 (nm)	分散性 (%)
1947	FM47 HA-Np	16.8	8.9
1954	MAL54 HA-Np	17.9	8.9
1957	DV57 HA-Np	17.7	6.2
1977	HK77 HA-Np	17.6	10.9
1999	NC99 HA-Np	17.3	8.6
2009	CA09 HA-Np	16.8	9.3
1999-2012	COBRA X6 HA-Np	17.5	5.1
1933-1957 + 2009-2011 和猪 1931-1998	COBRA P1 HA-Np	17.1	7

图18A

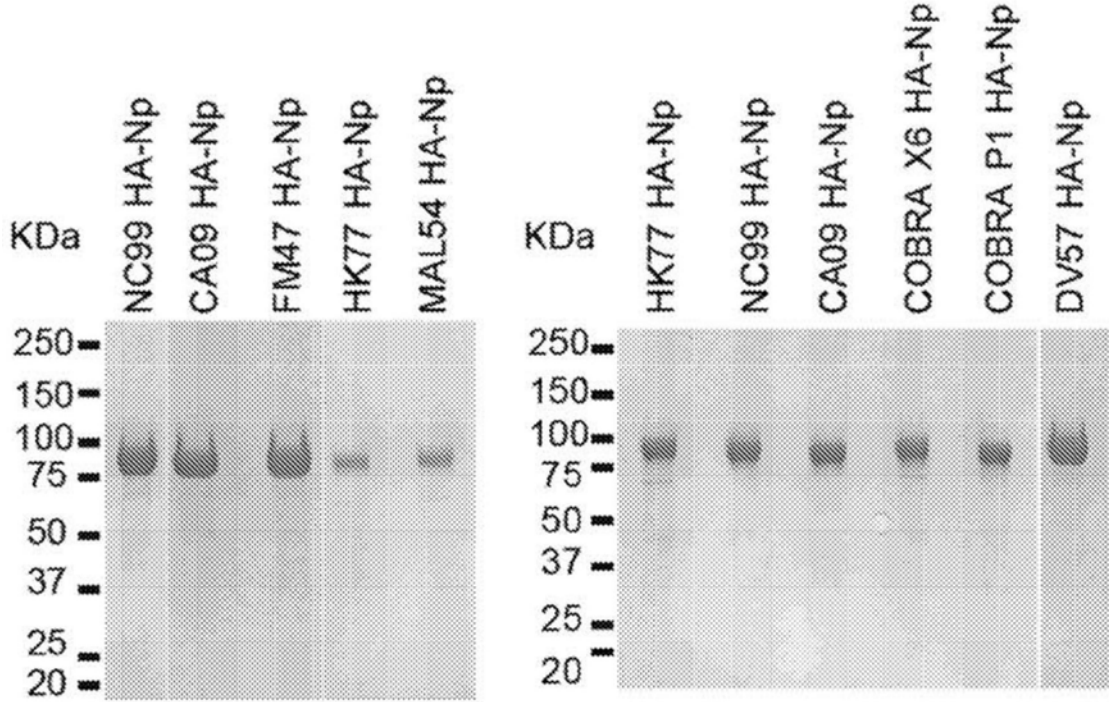


图18B

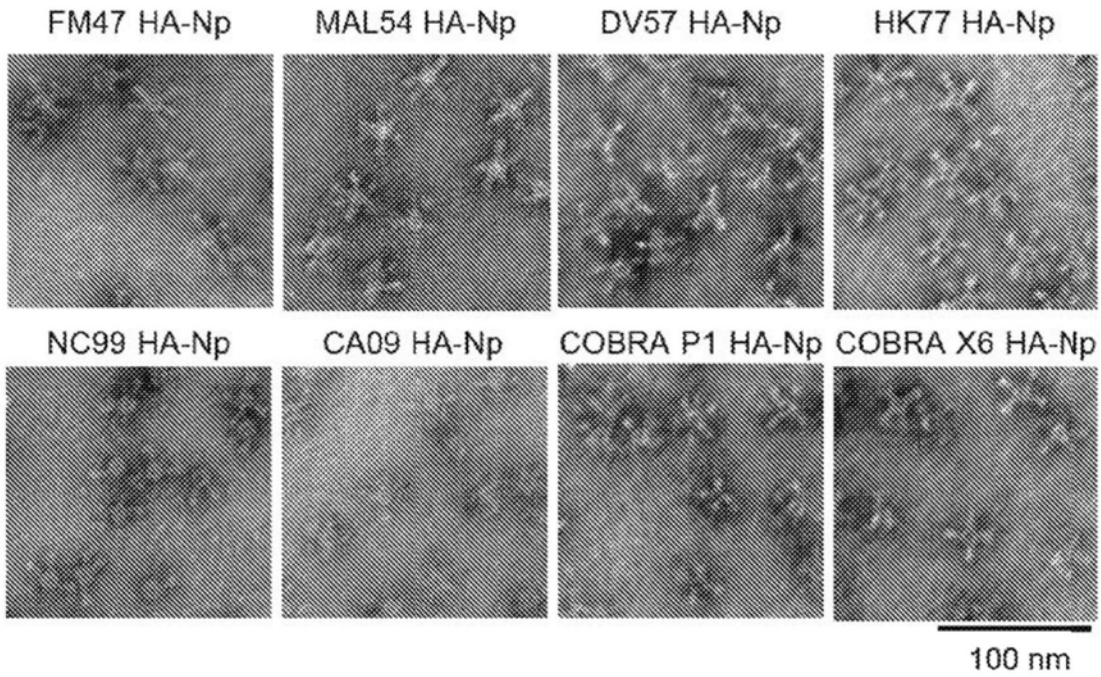


图18C

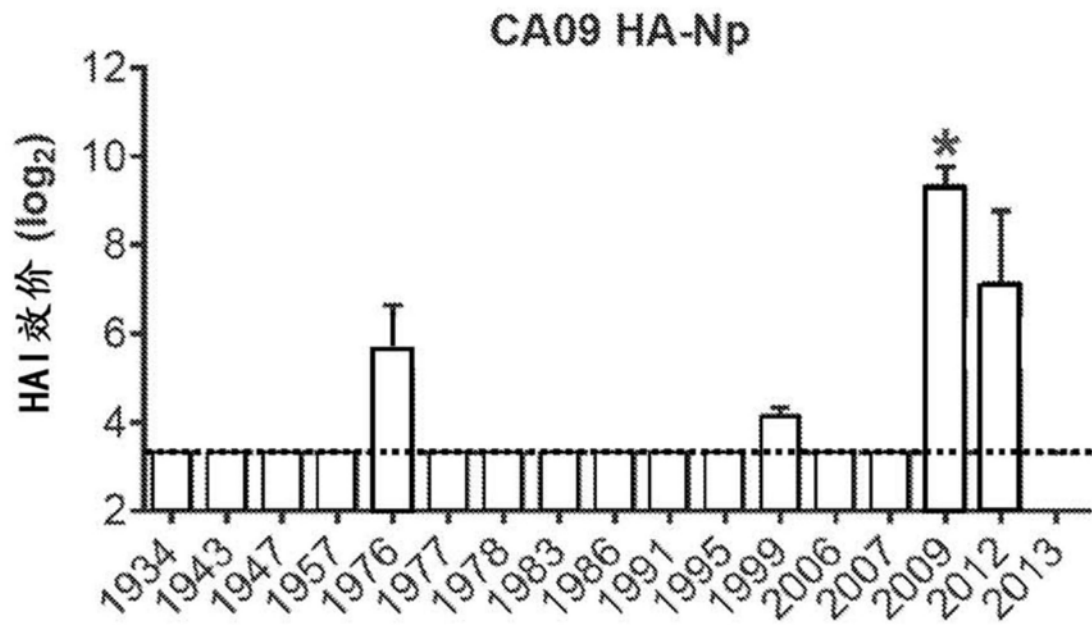


图19A

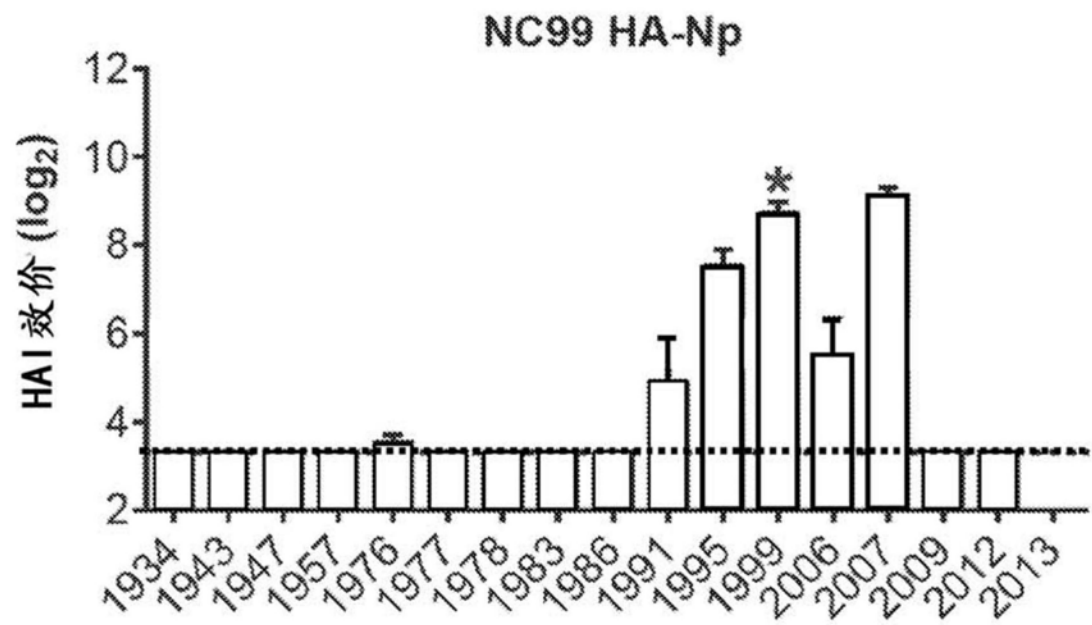


图19B

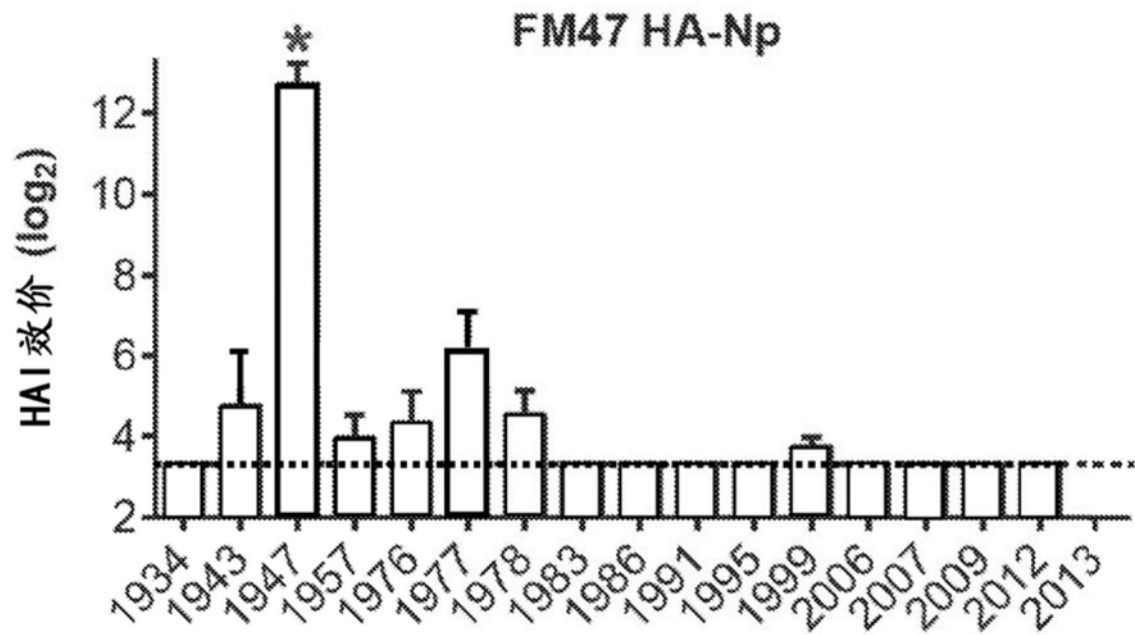


图19C

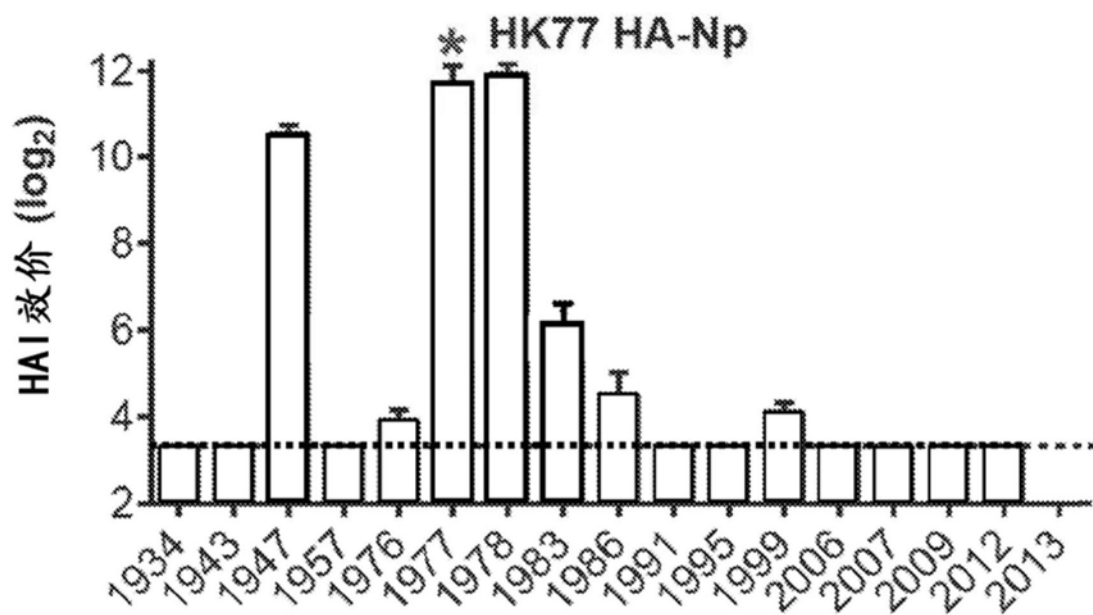


图19D

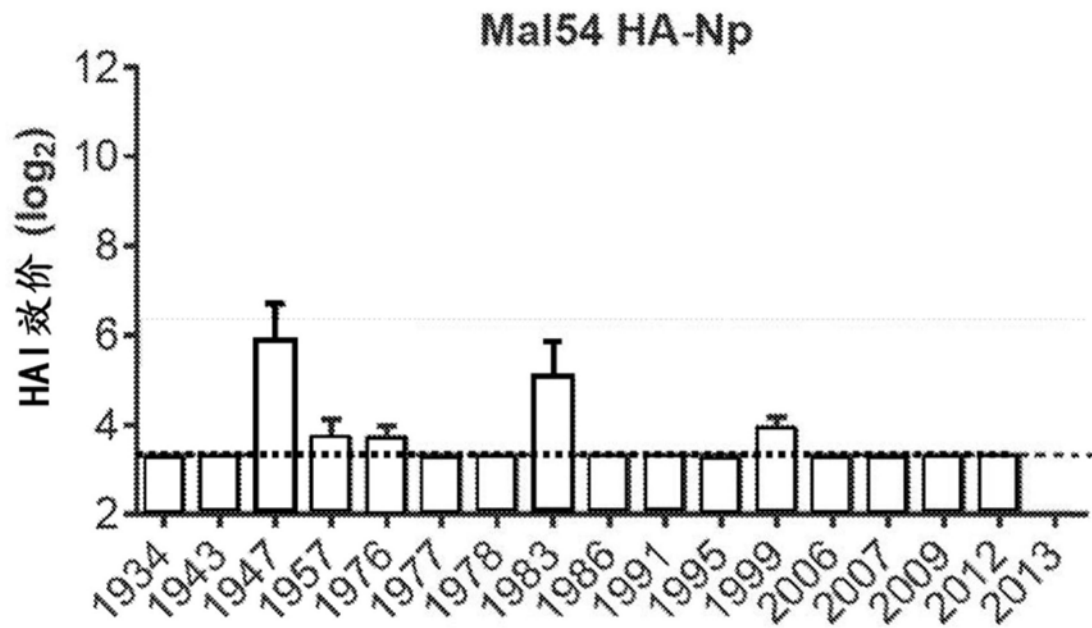


图19E

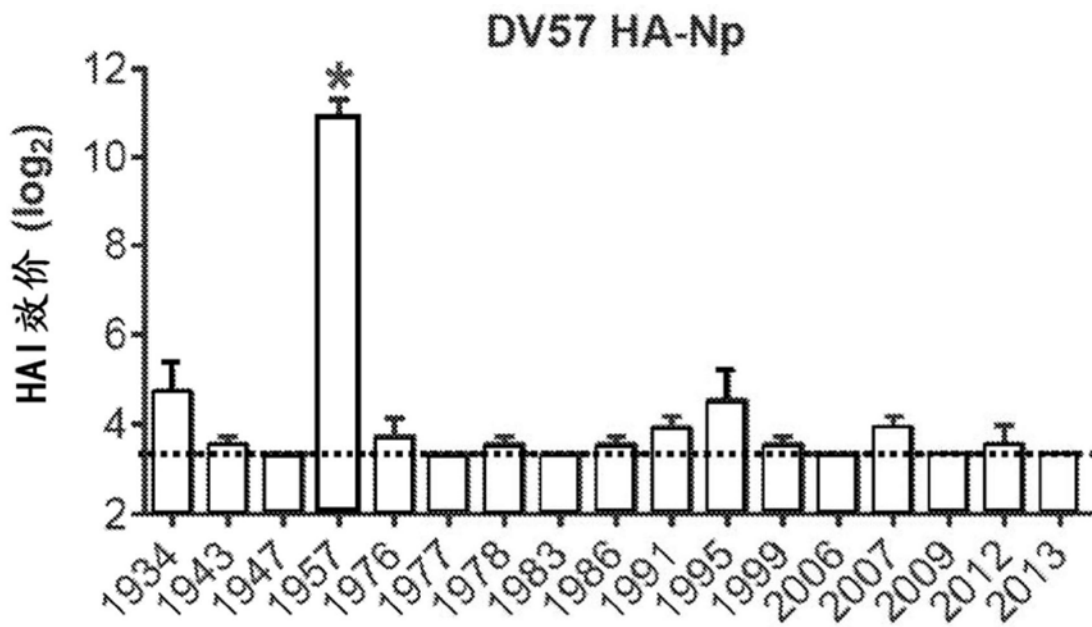


图19F

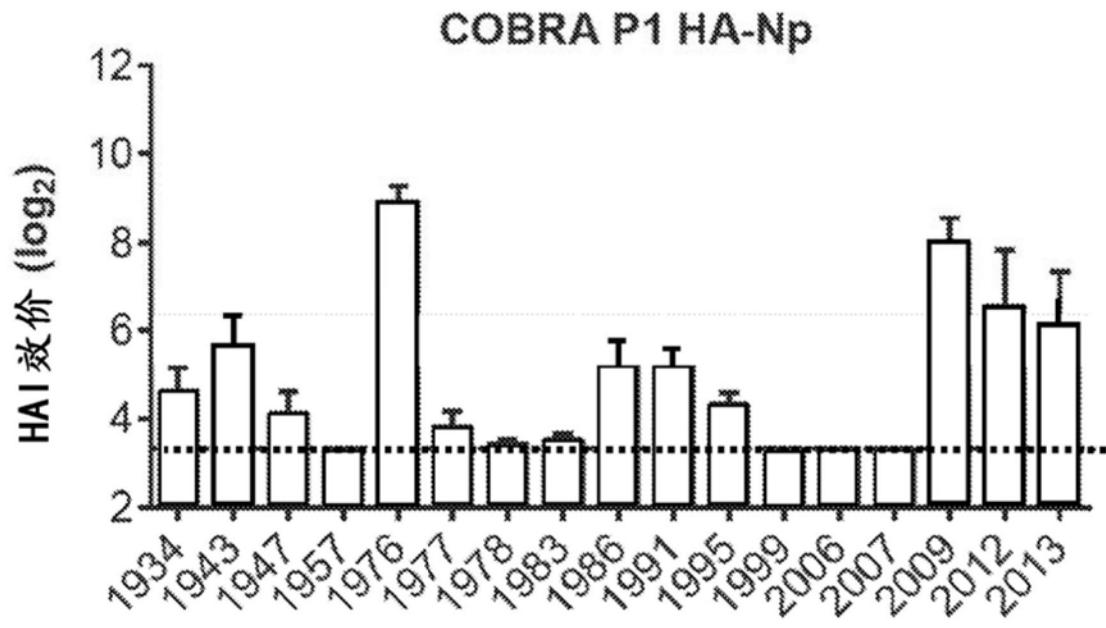


图19G

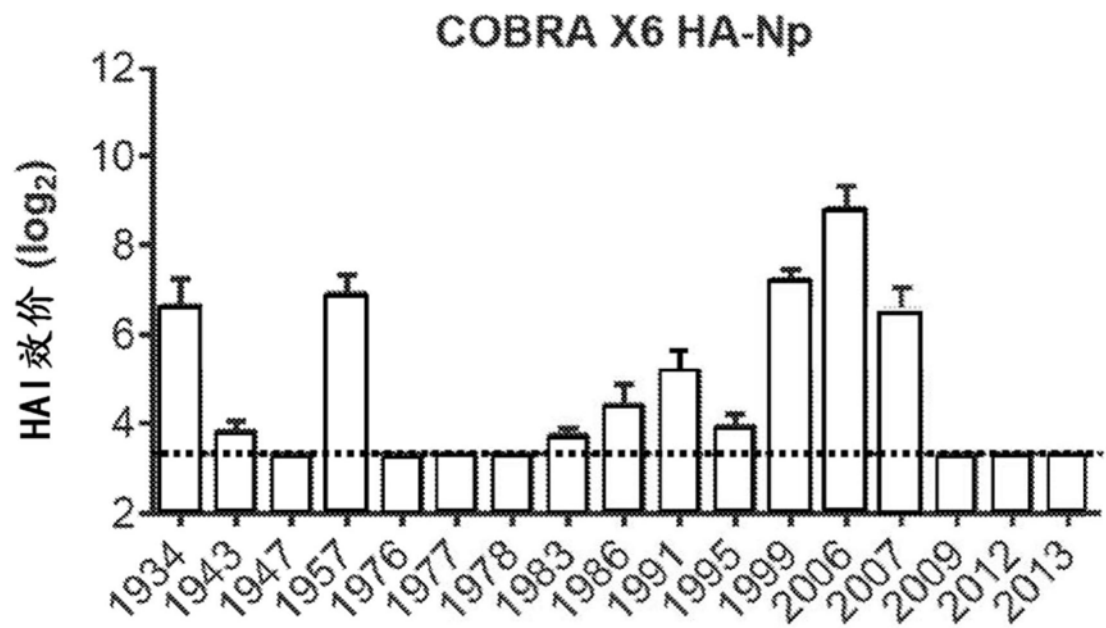


图19H

#	免疫原
1	NC99 HA-Np
2	CA09 HA-Np
3	FM47 HA-Np
4	HK77 HA-Np
5	MAL54 HA-Np
6	NC99 + CA09 HA-Nps
7	NC99 + CA09 + FM47 HA-Nps
8	NC99 + CA09 + HK77 HA-Nps
9	NC99 + CA09 + MAL54 HA-Nps
10	NC99 + CA09 + HK77 + FM47 HA-Nps
11	NC99 + CA09 + HK77 + MAL54 HA-Nps
12	NC99 + CA09 + FM47 + MAL54 HA-Nps
13	NC99 IIV
14	CA09 IIV
15	NC99 + CA09 IIV

图20A

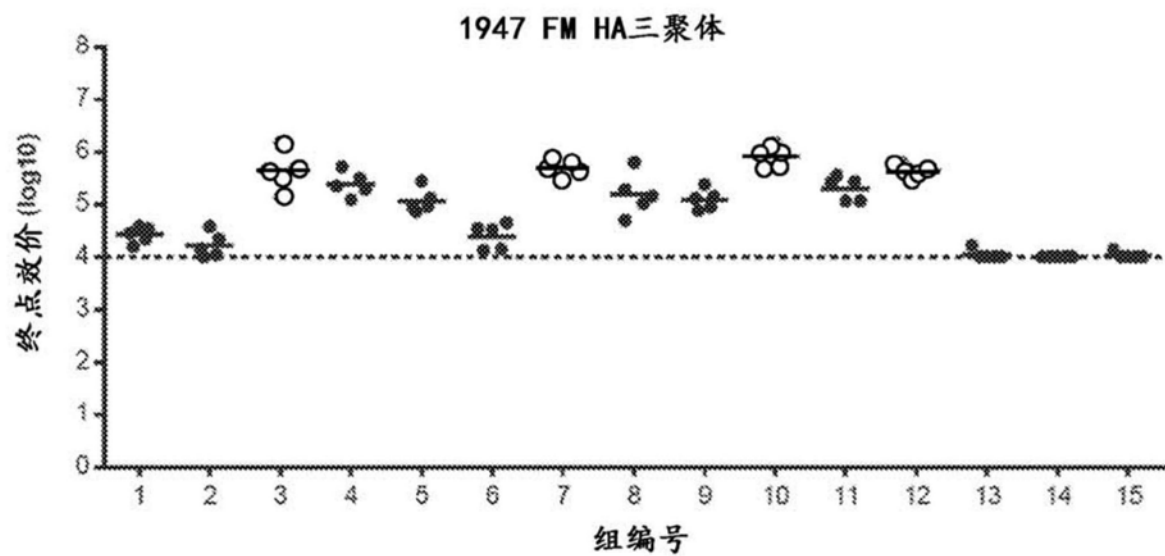


图20B

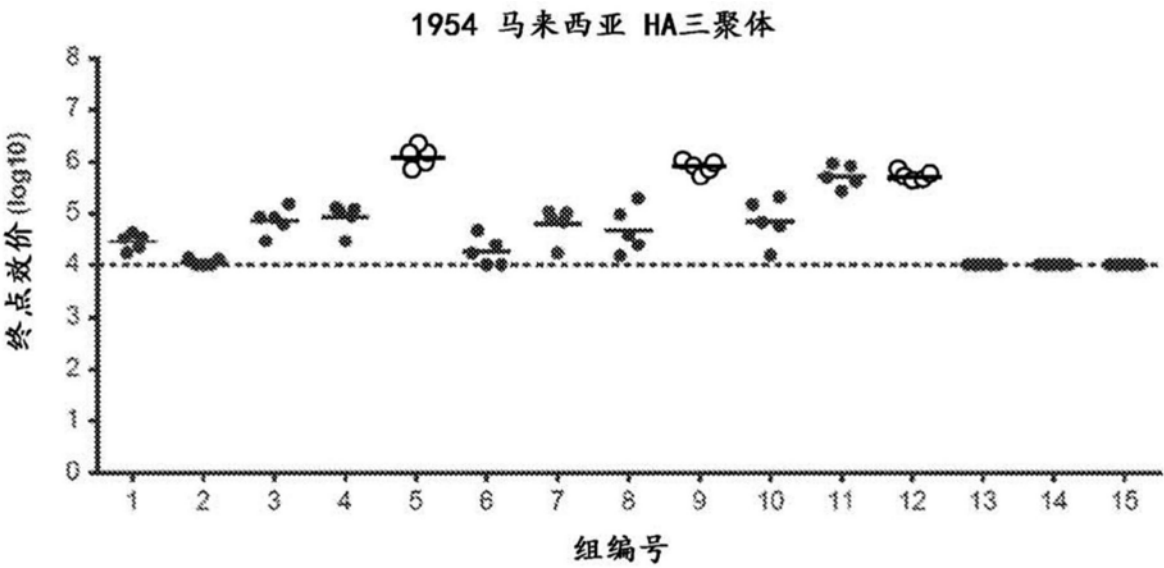


图20C

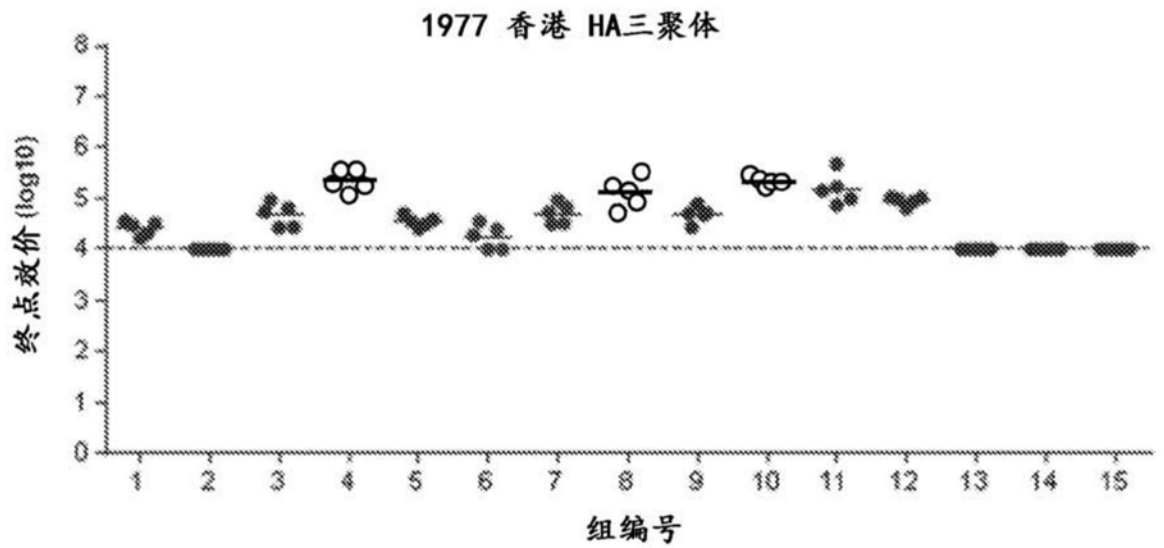


图20D

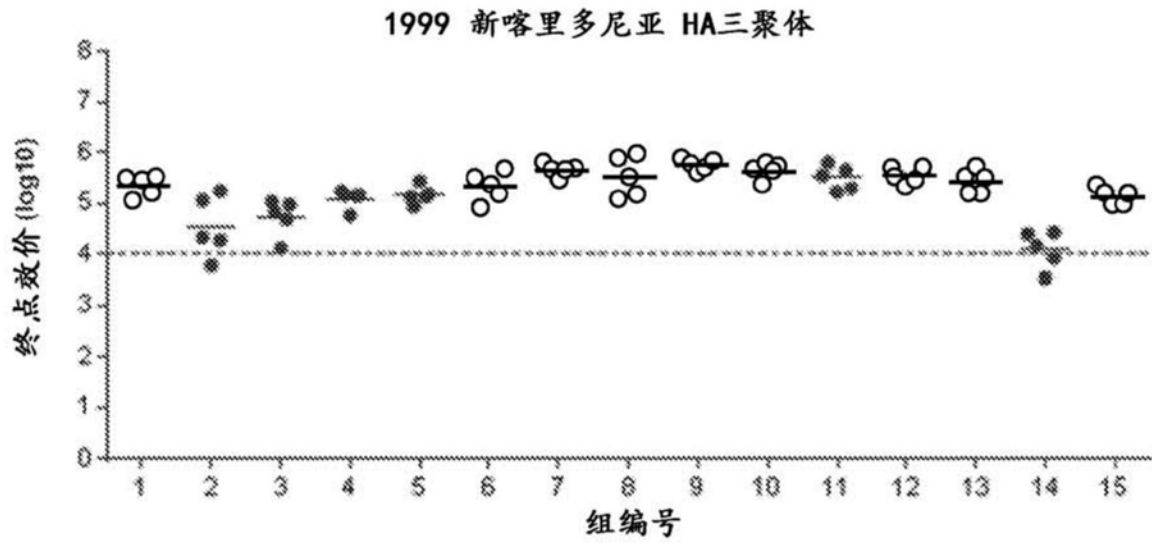


图20E

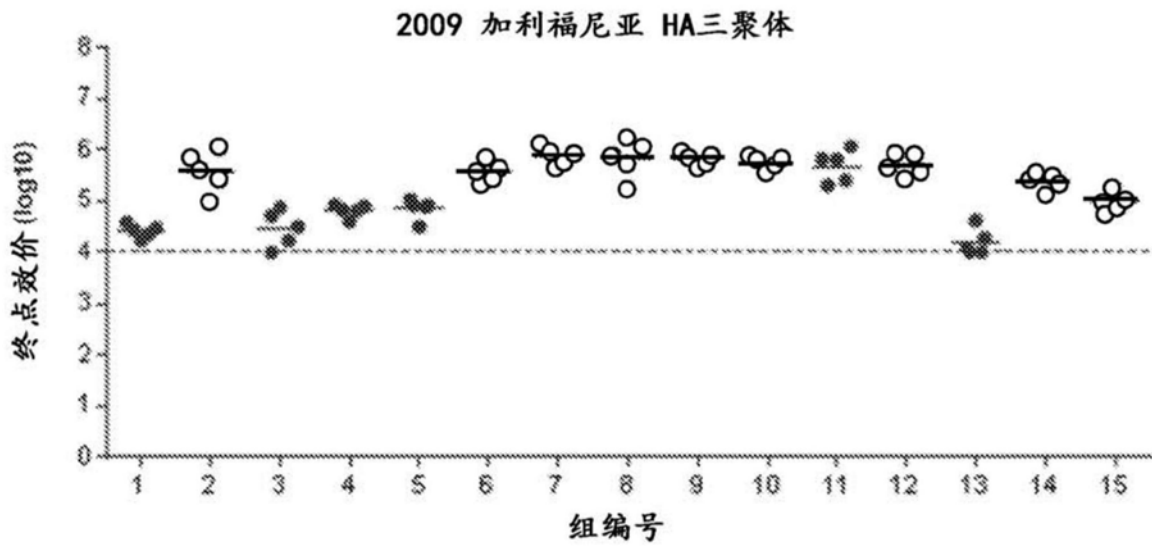


图20F

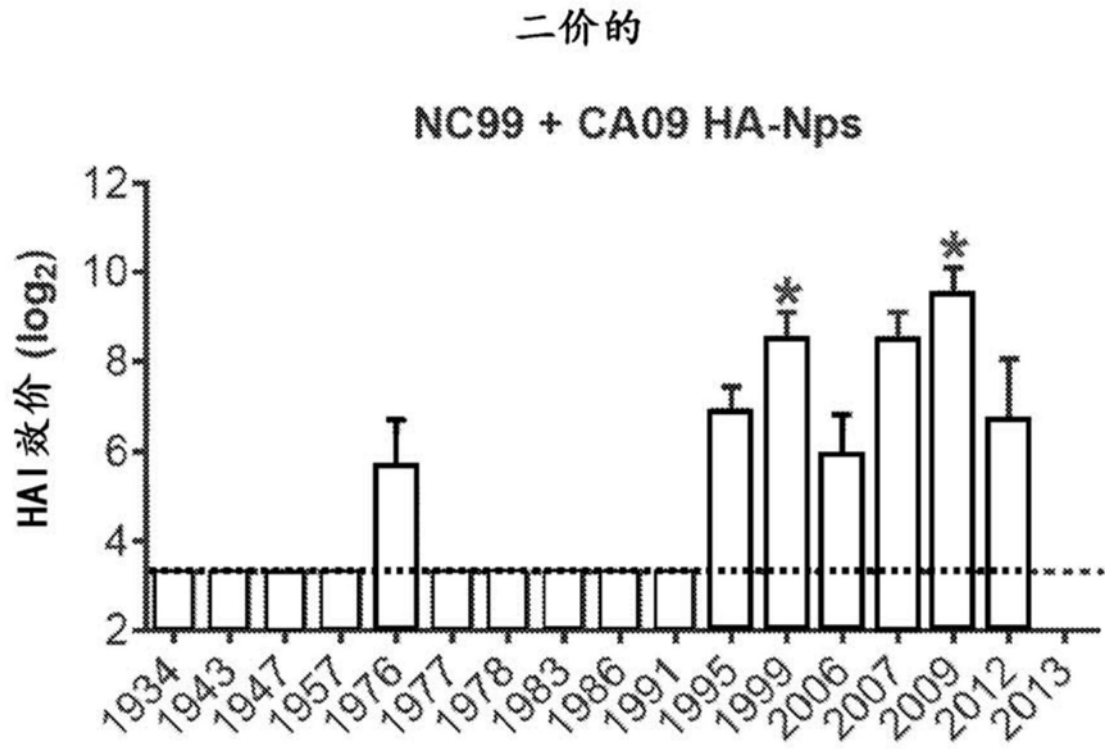


图21A

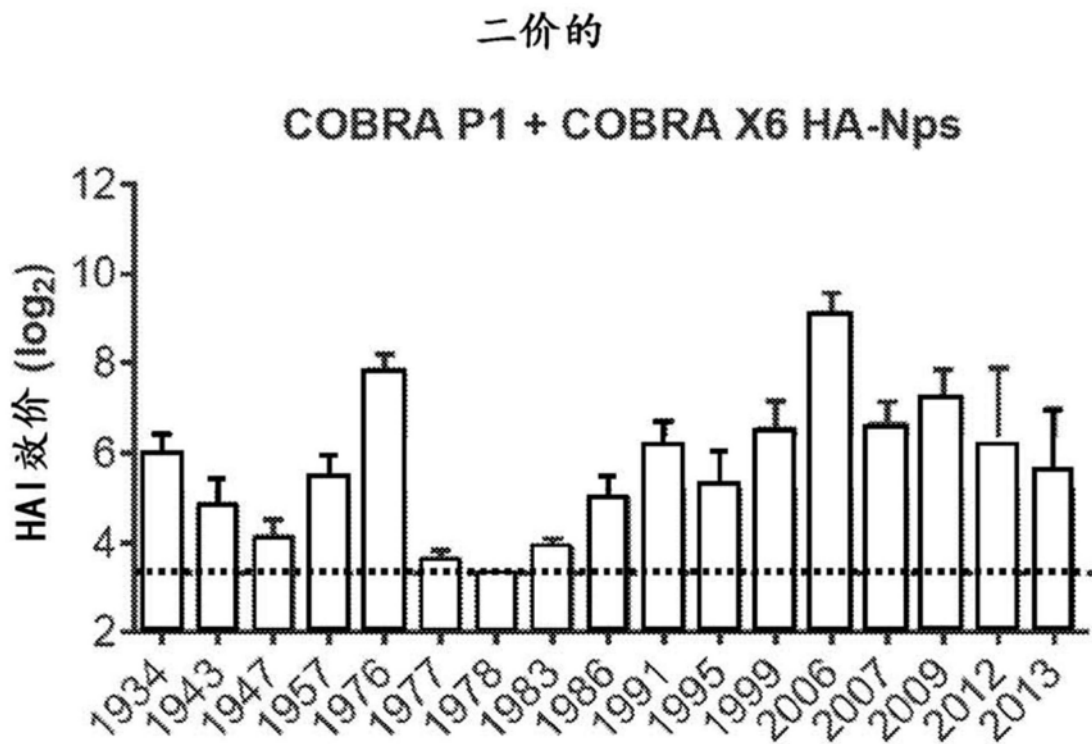


图21B

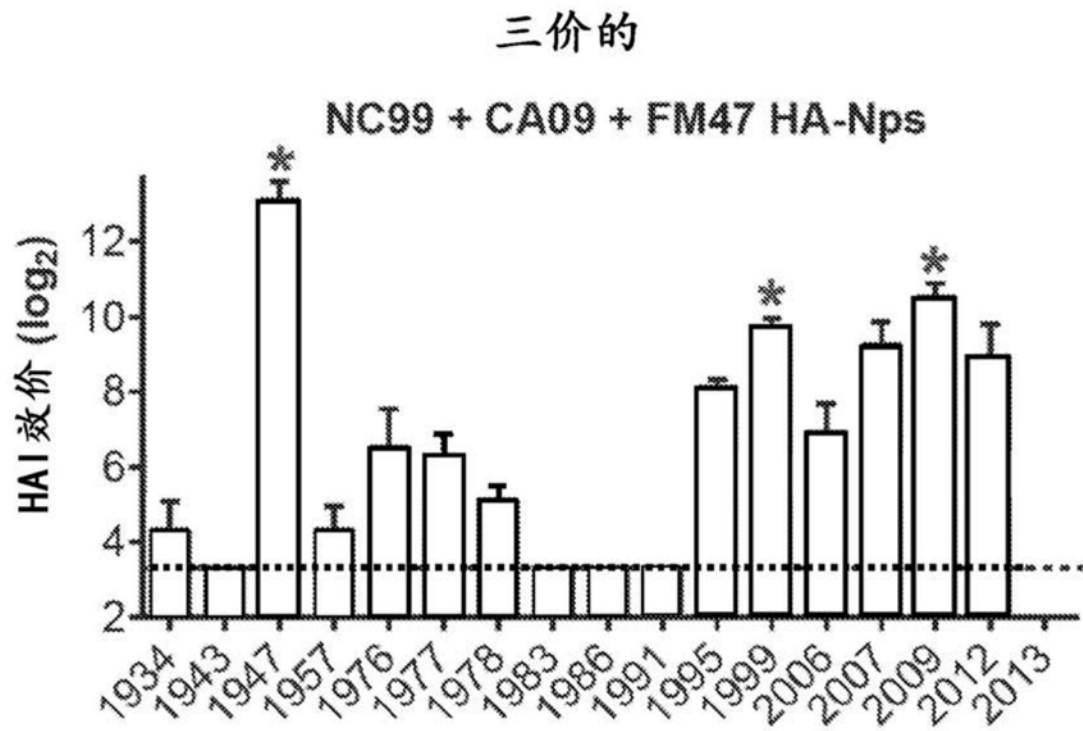


图21C

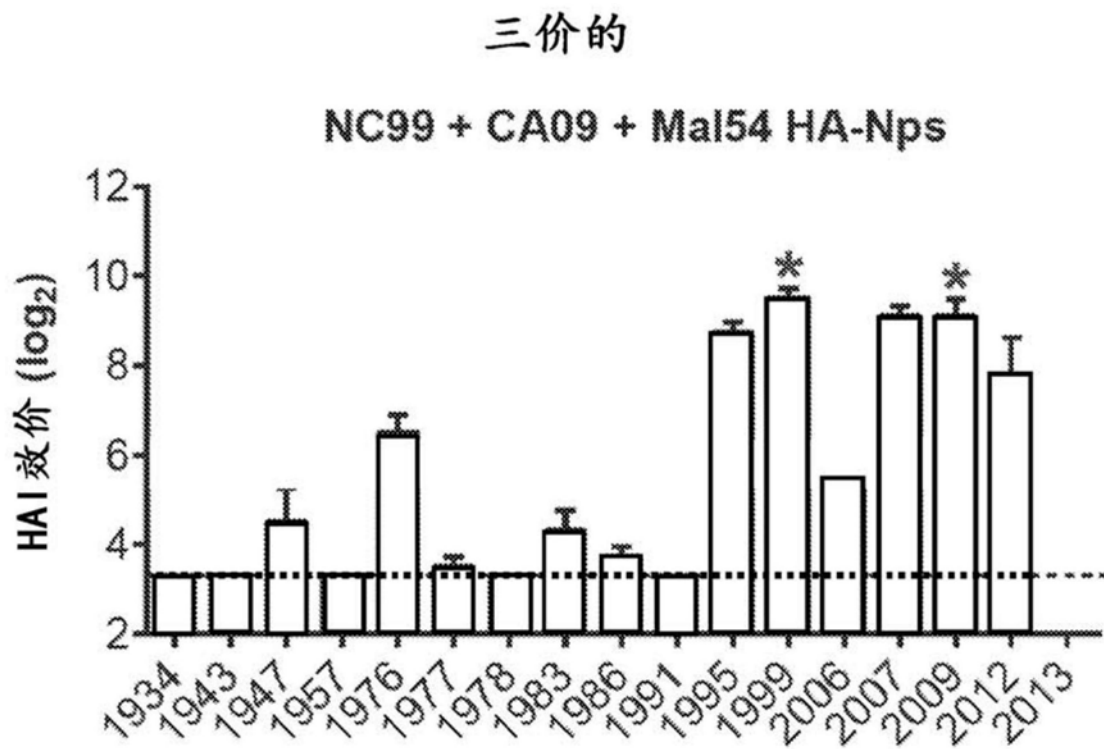


图21D

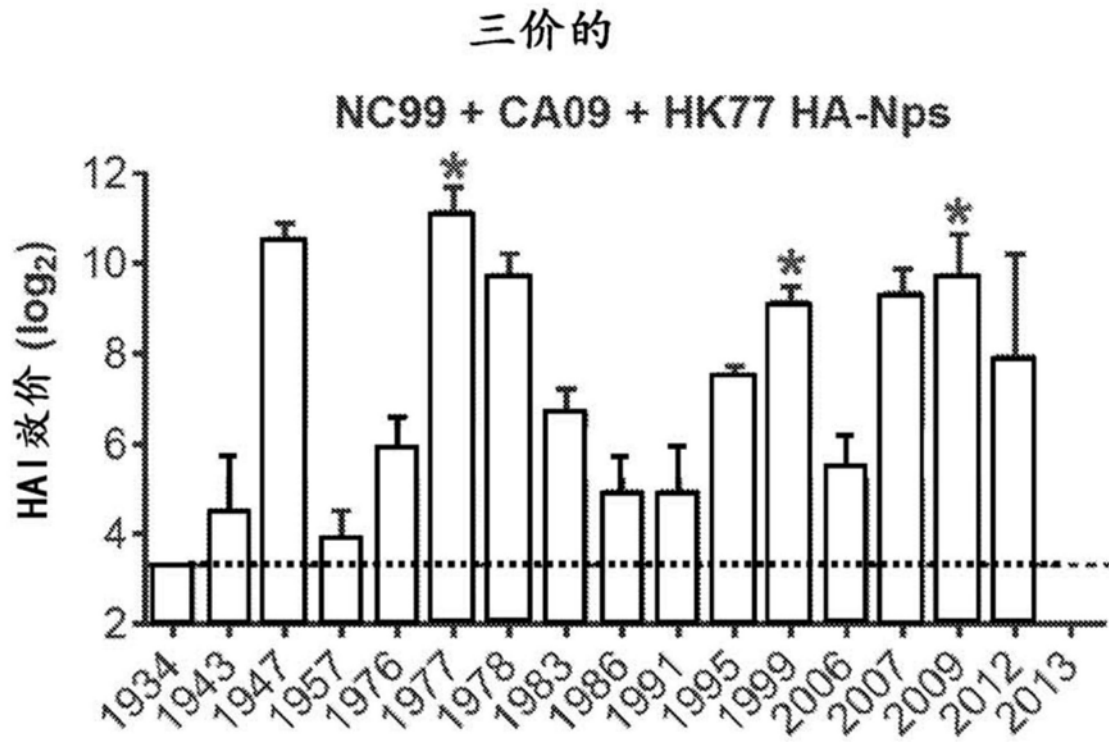


图21E

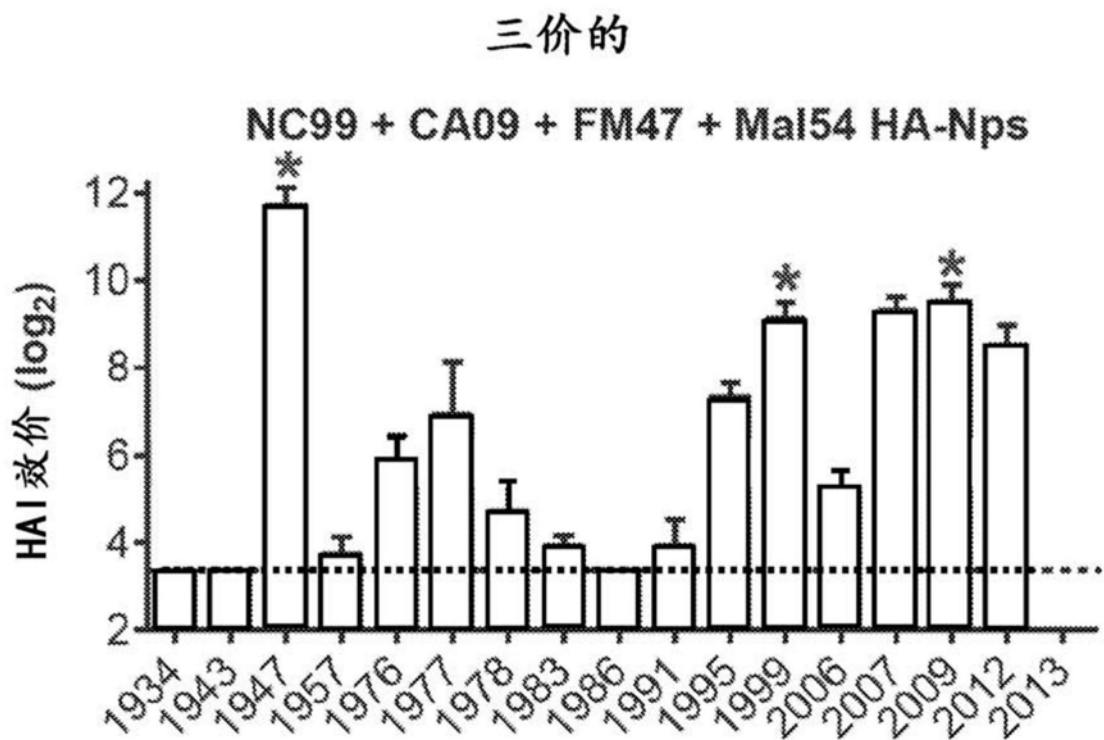


图21F

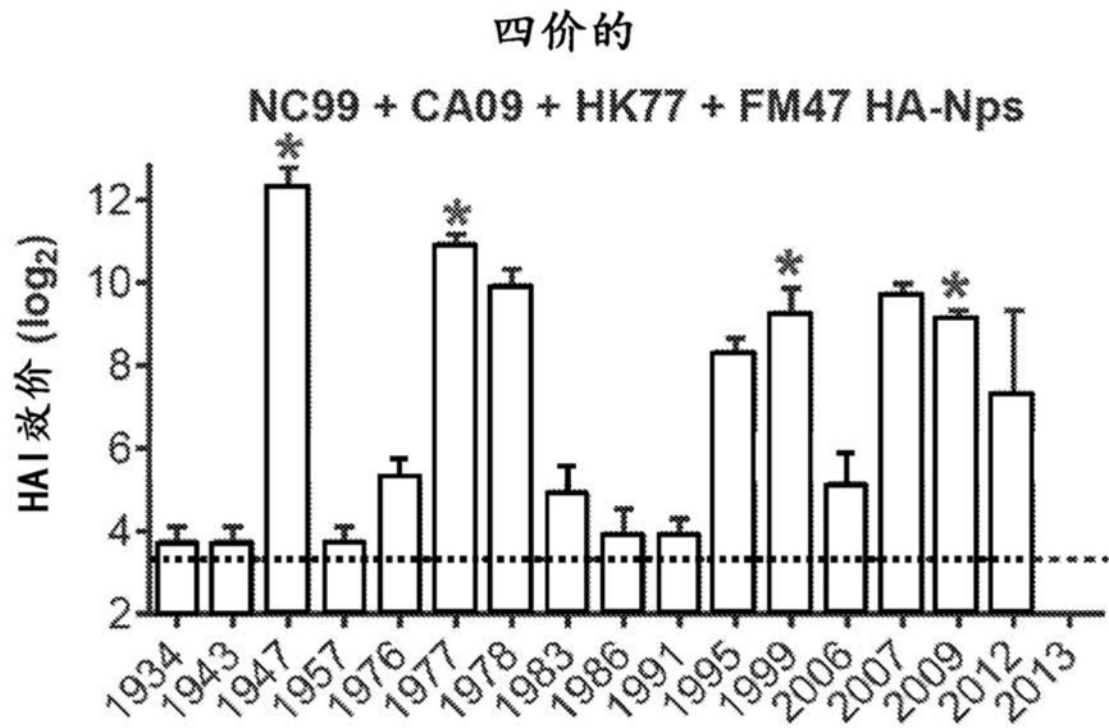


图21G

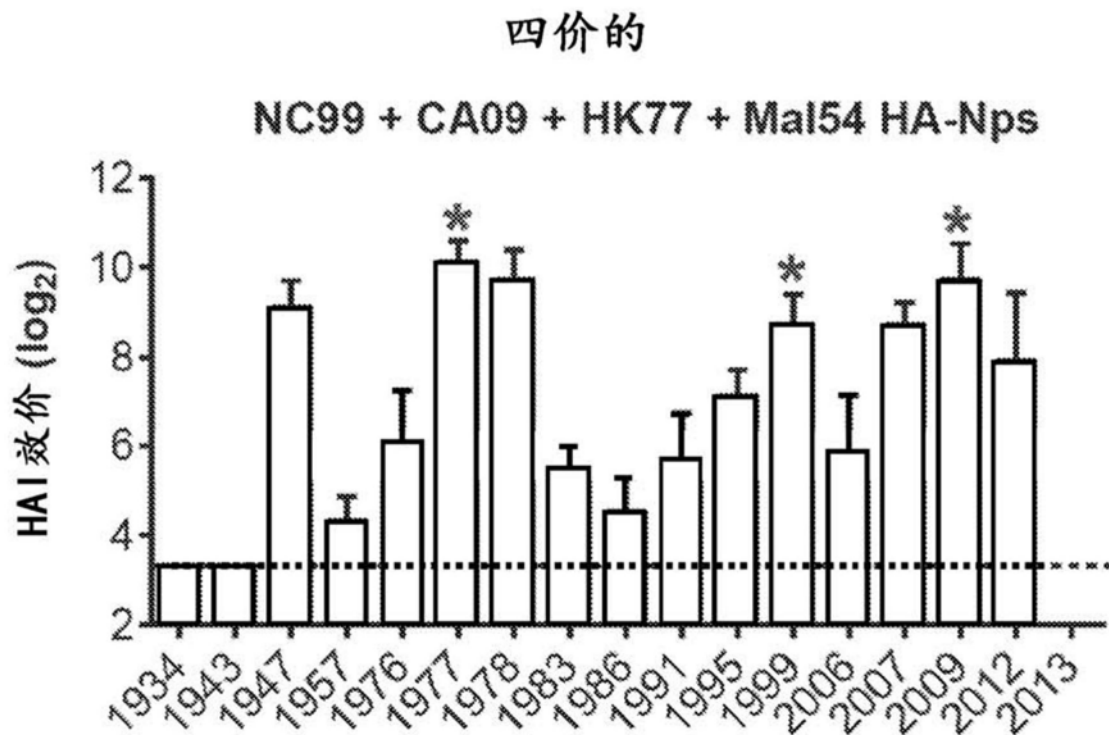


图21H

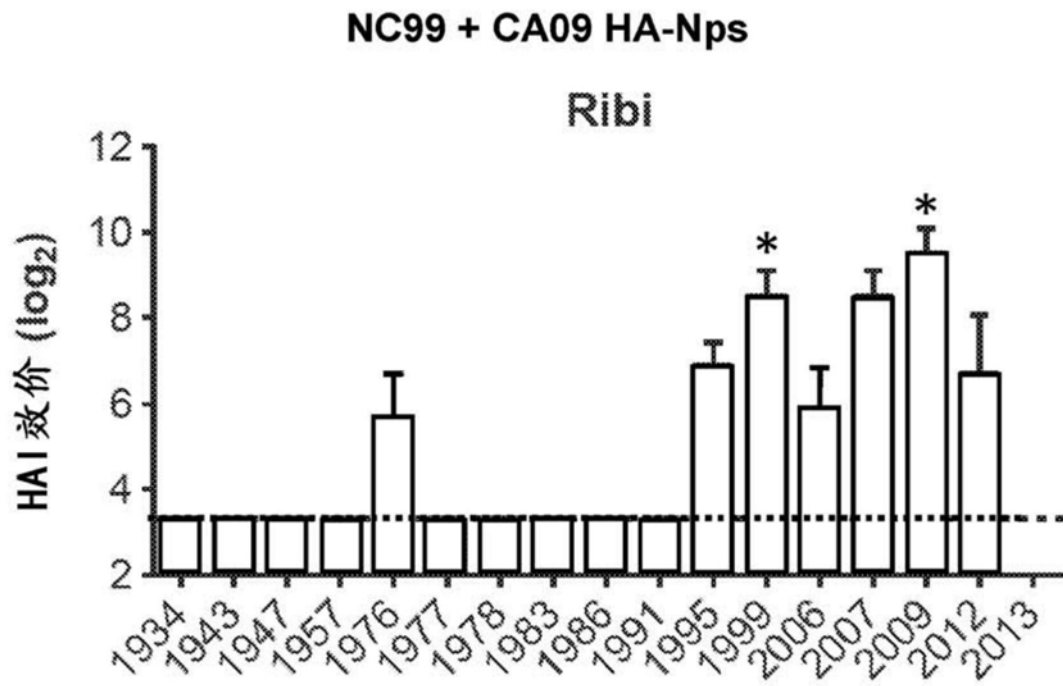


图22A

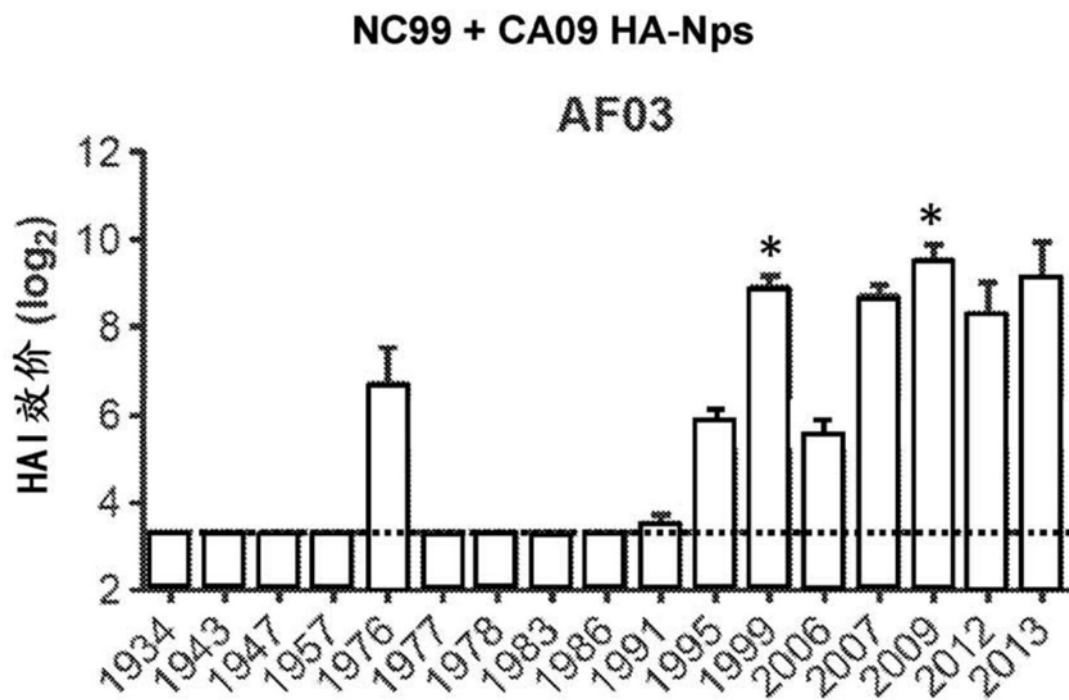


图22B

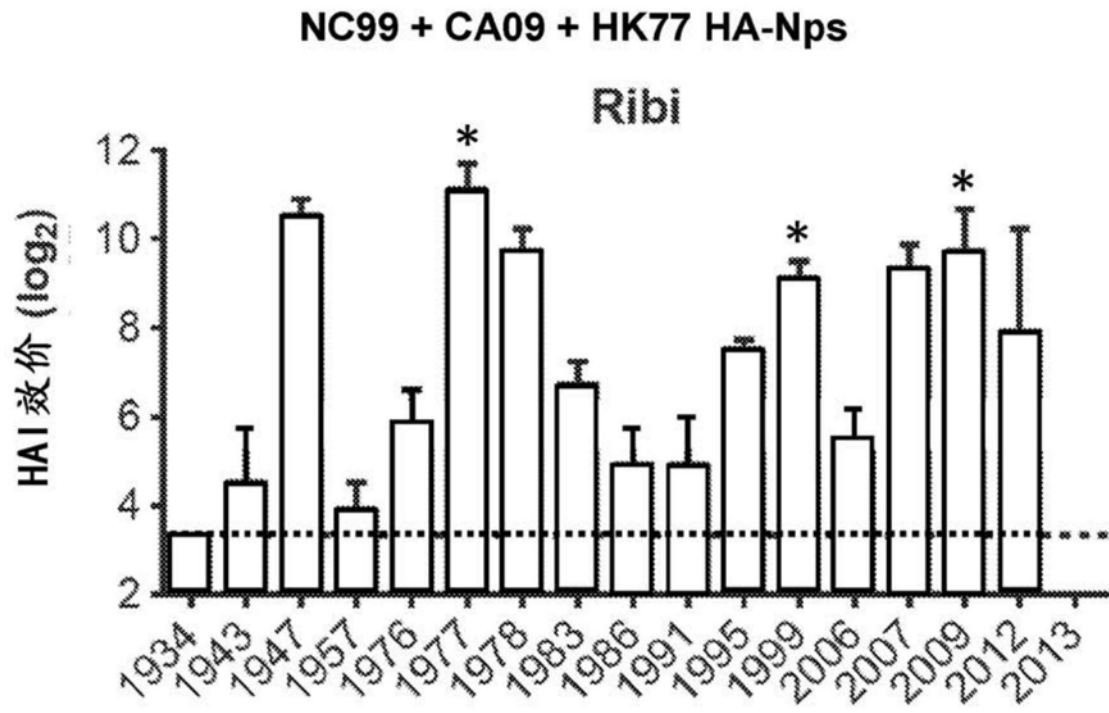


图22C

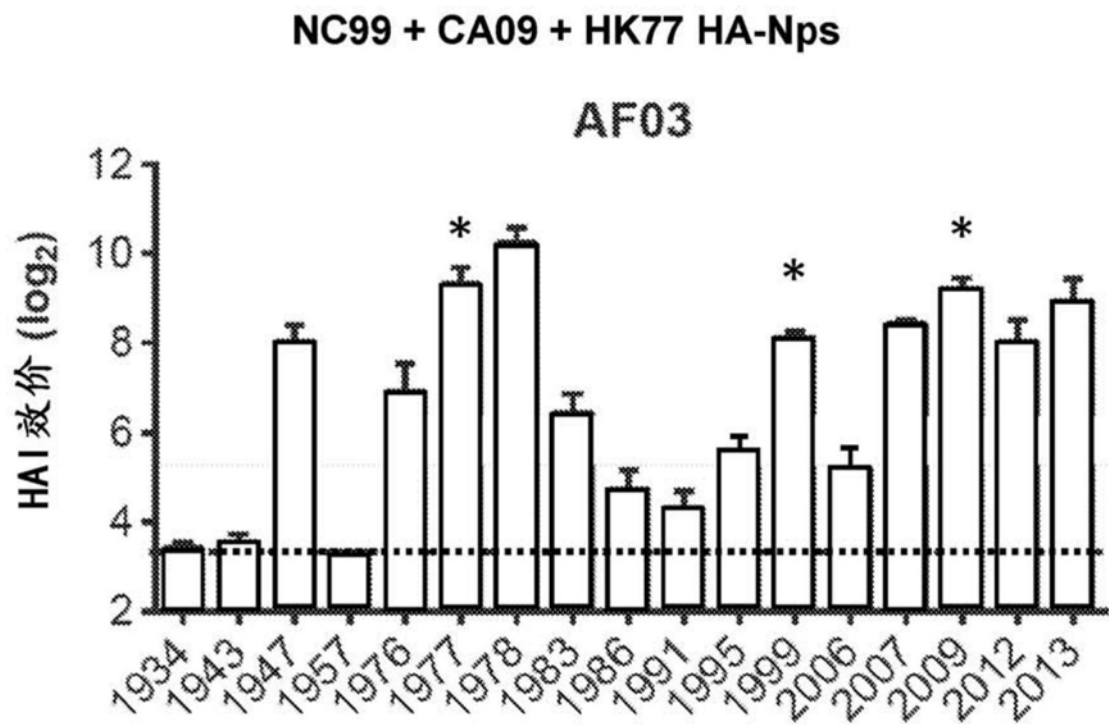


图22D

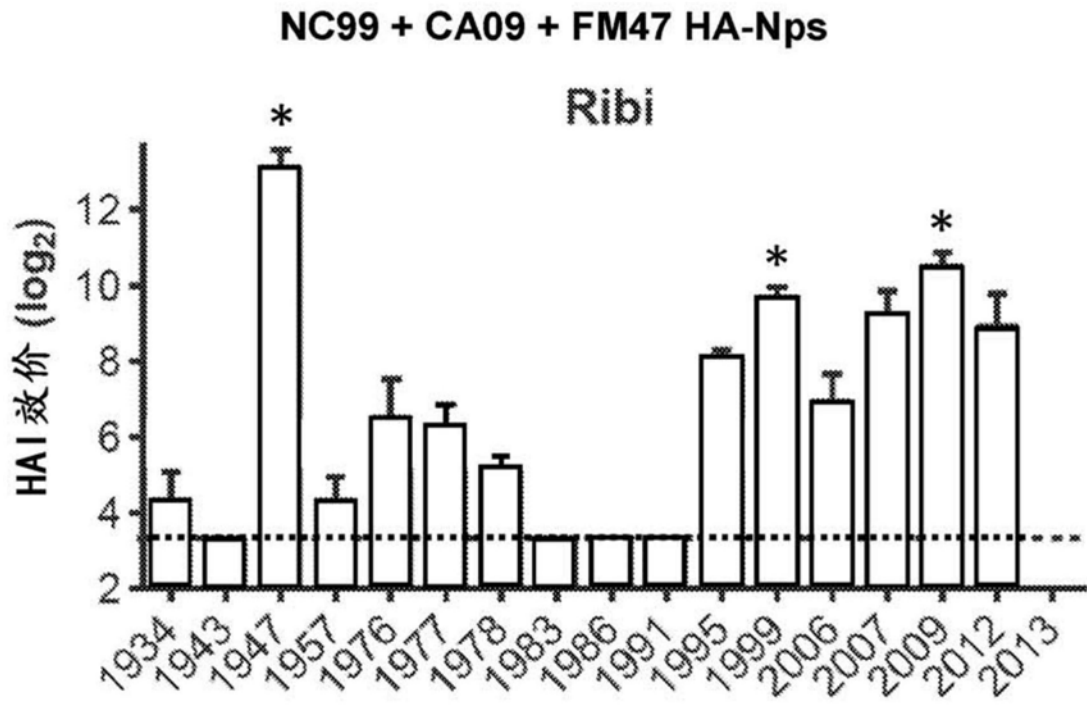


图22E

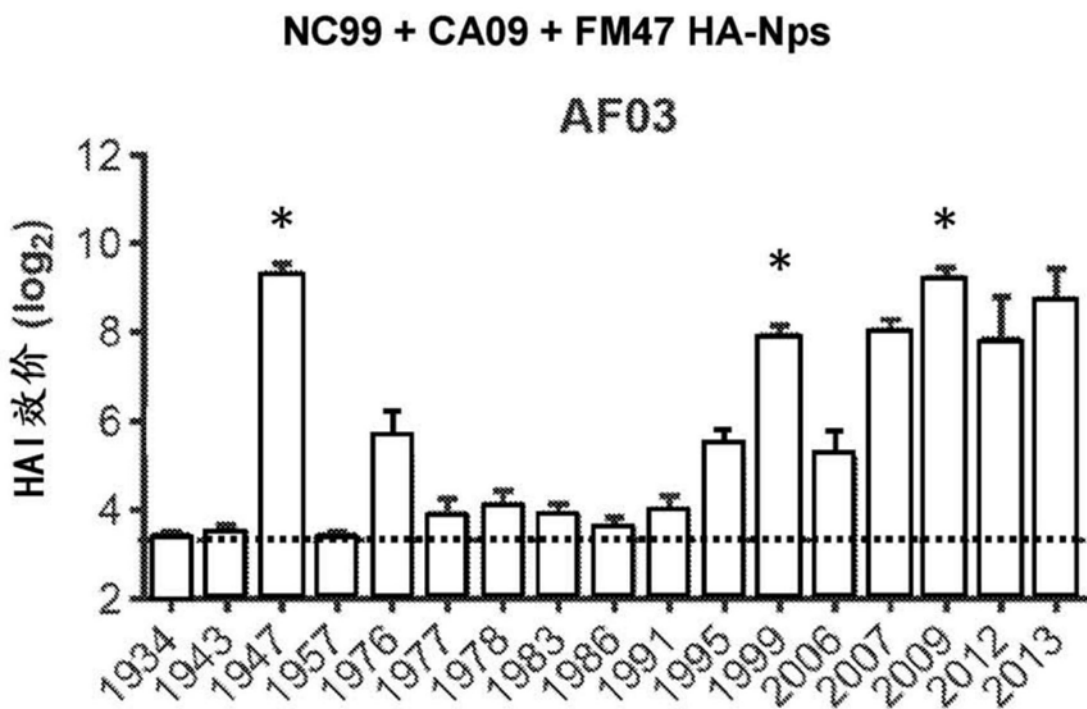


图22F

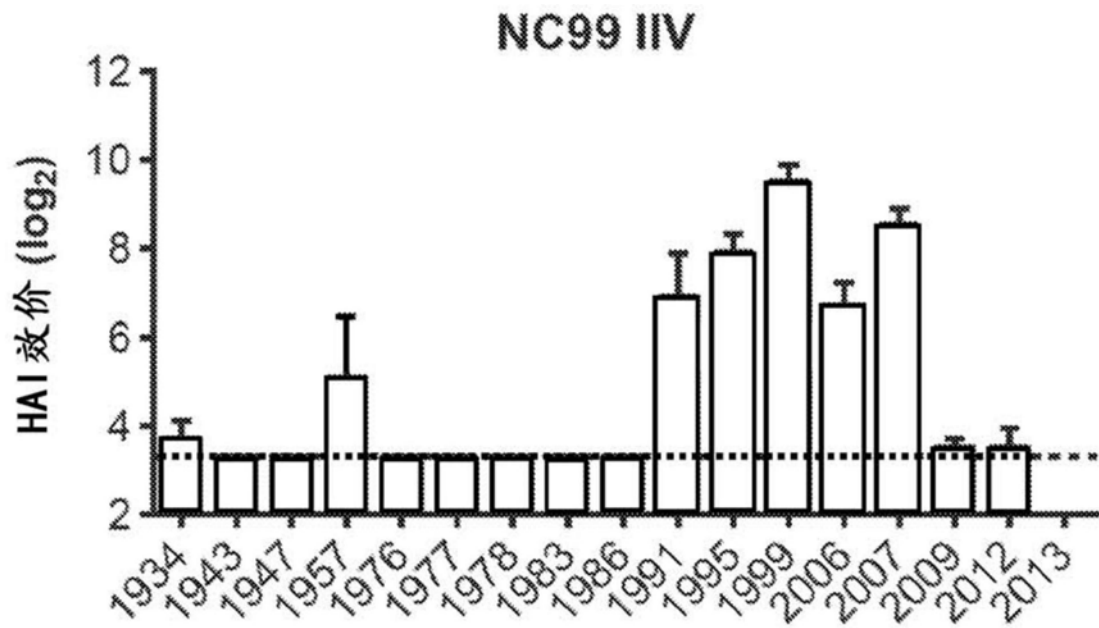


图23A

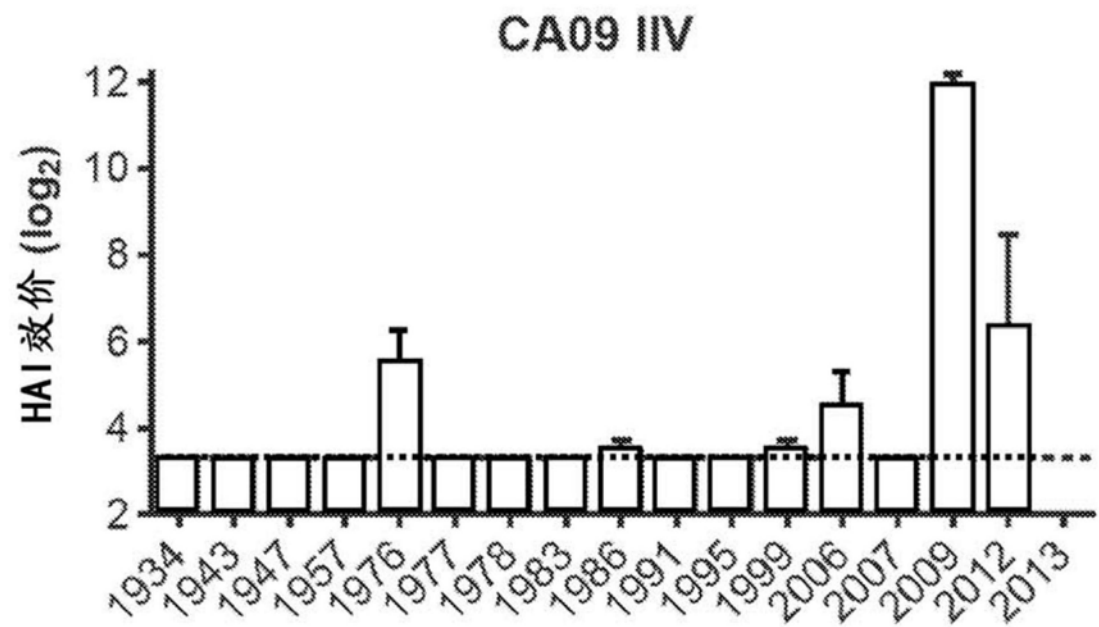


图23B

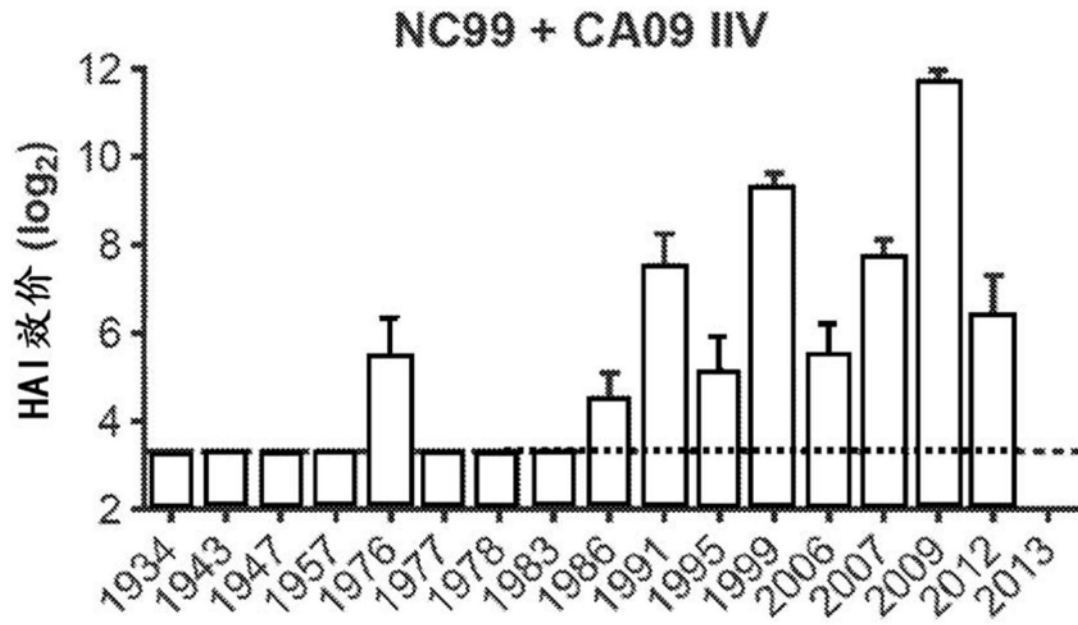


图23C

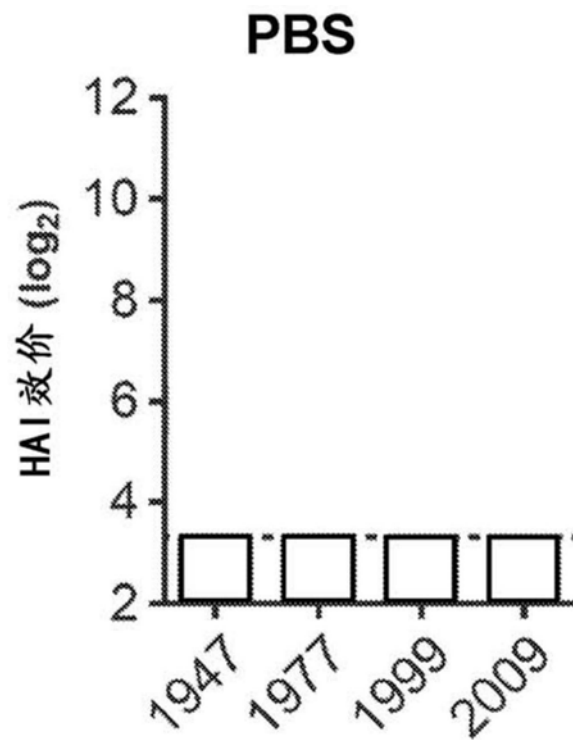


图24A

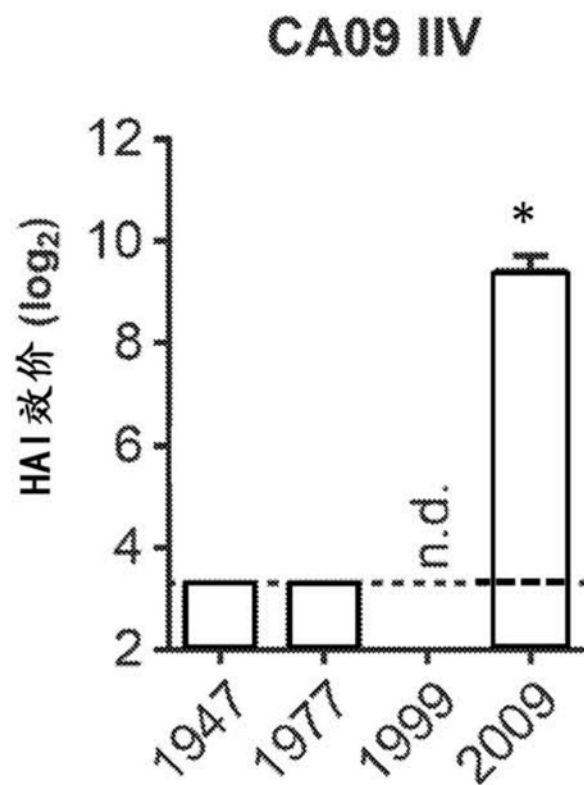


图24B

NC99 + CA09 + HK77 HA-Nps

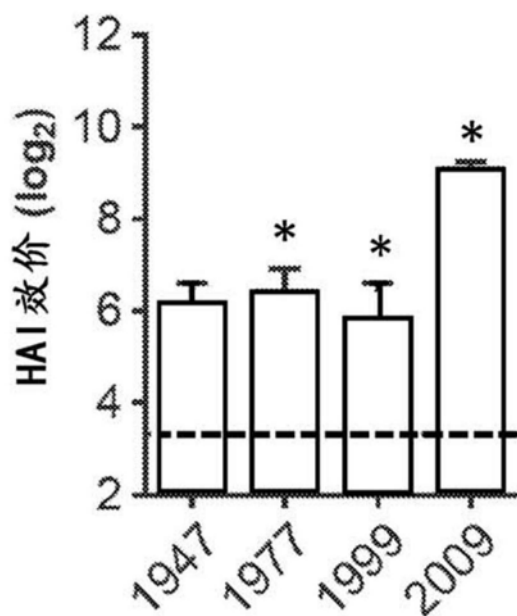


图24C

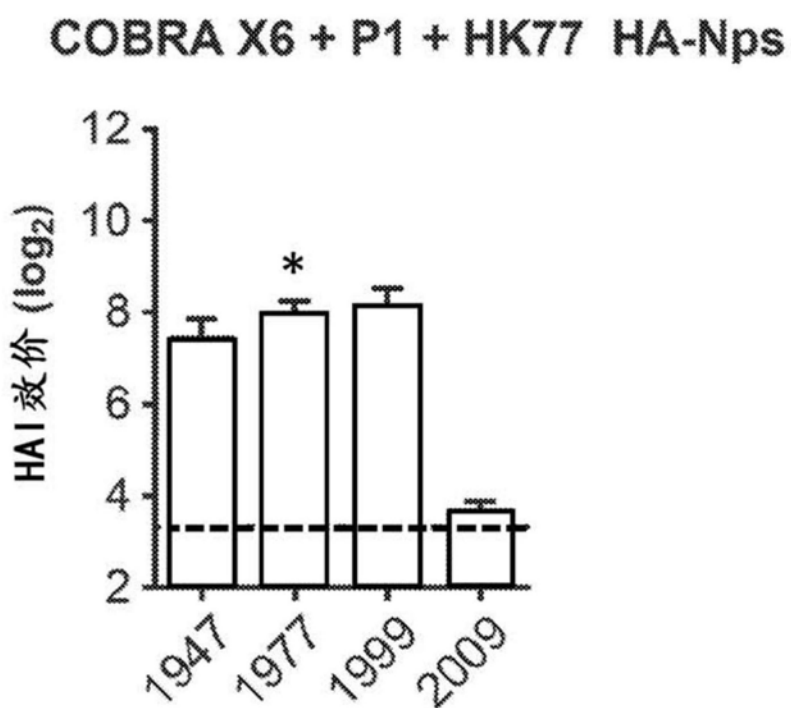


图24D

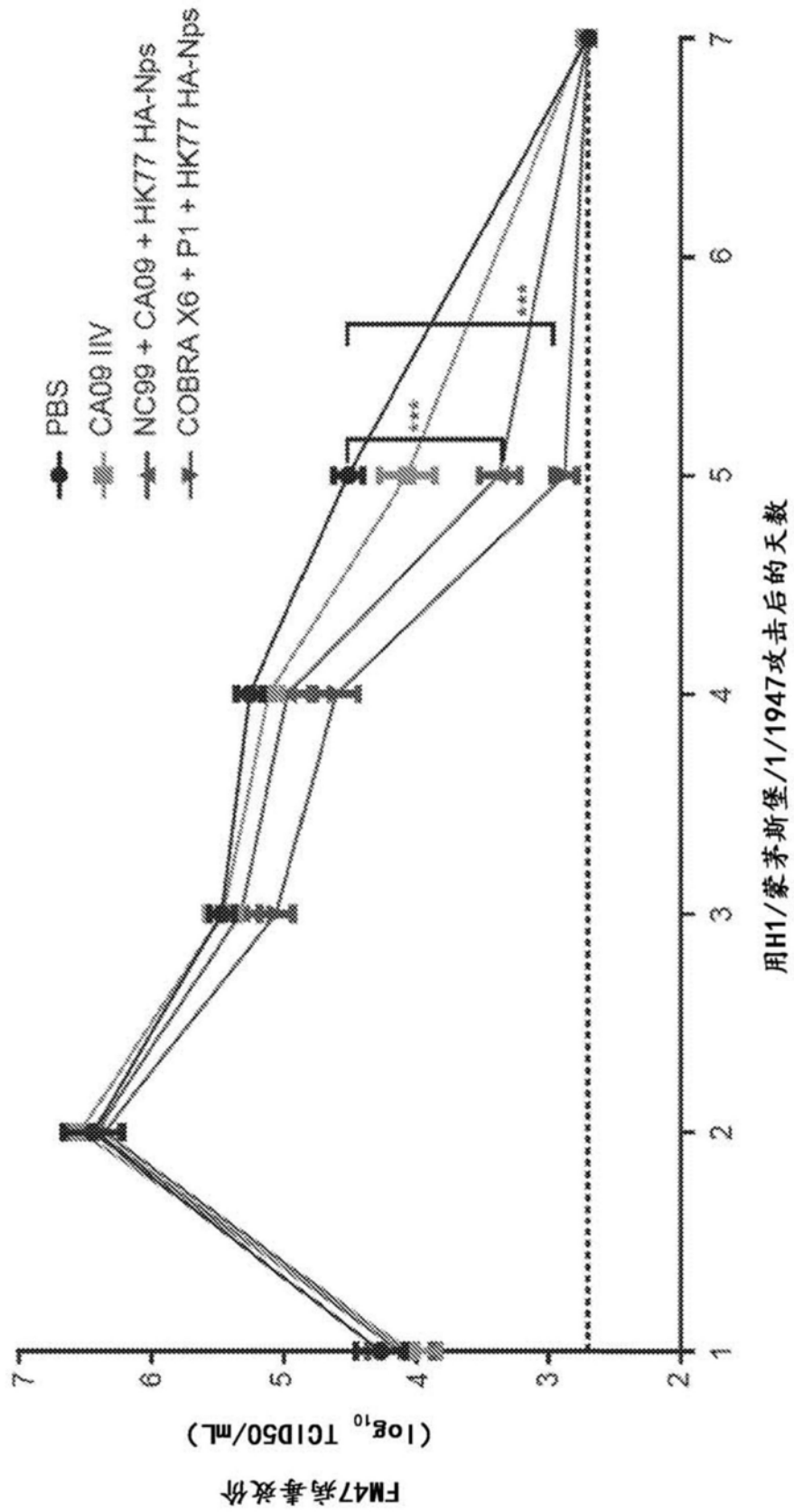


图24E

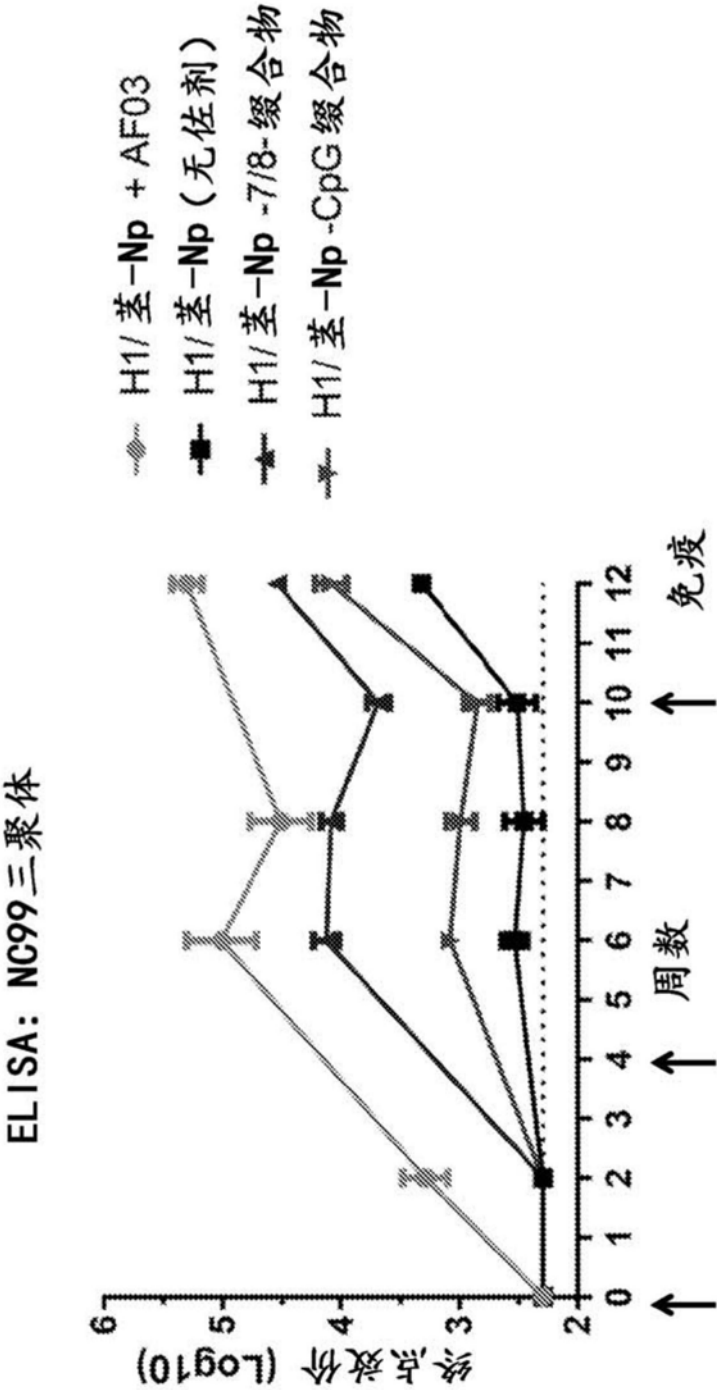


图25A

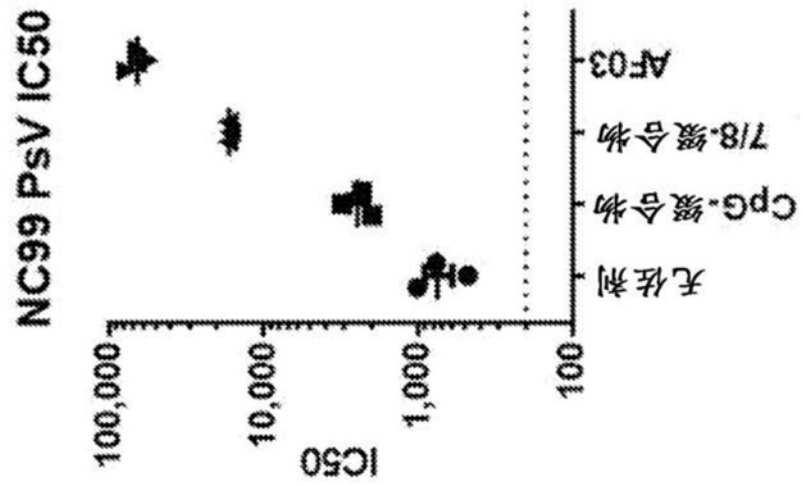


图25B

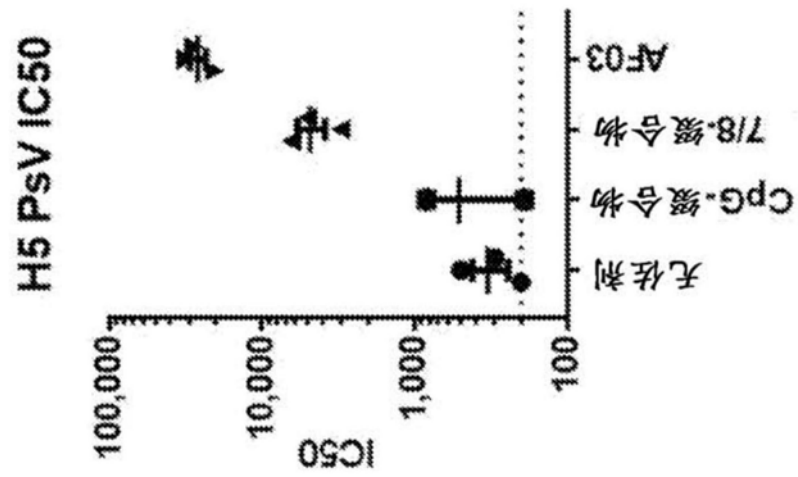


图25C

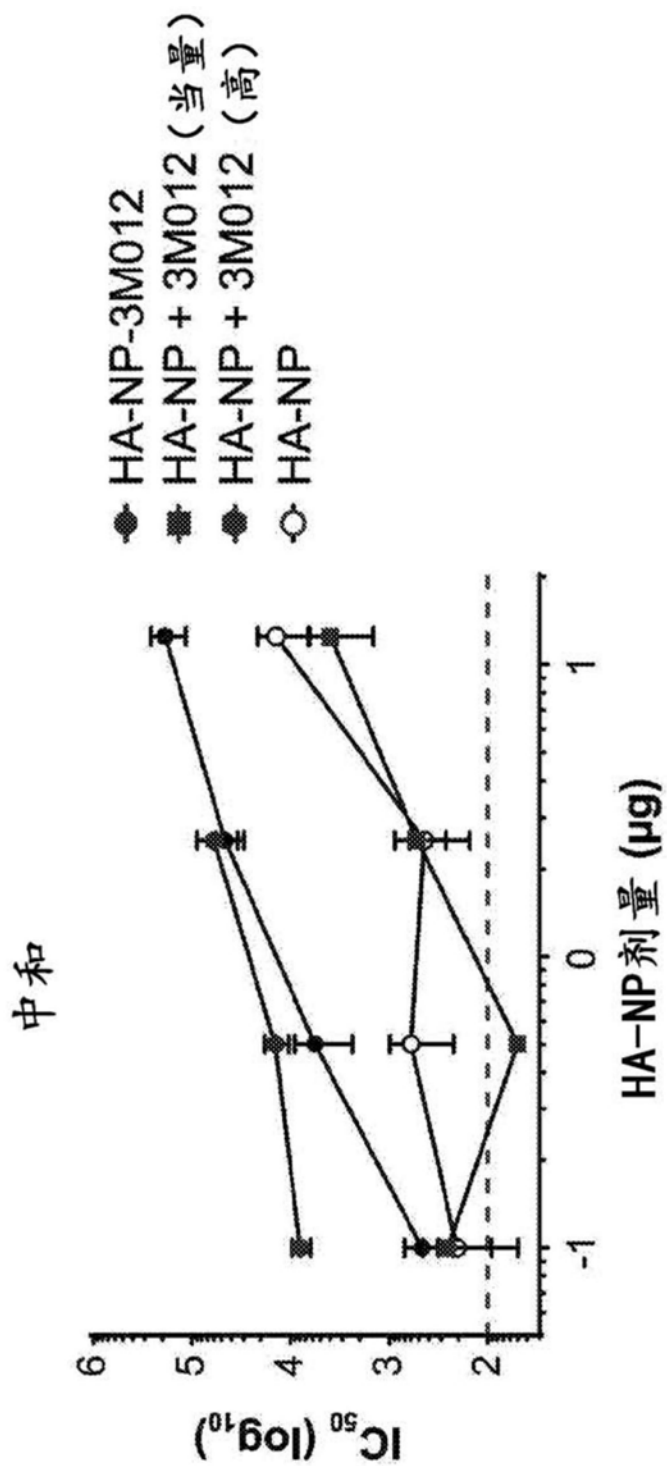


图26A

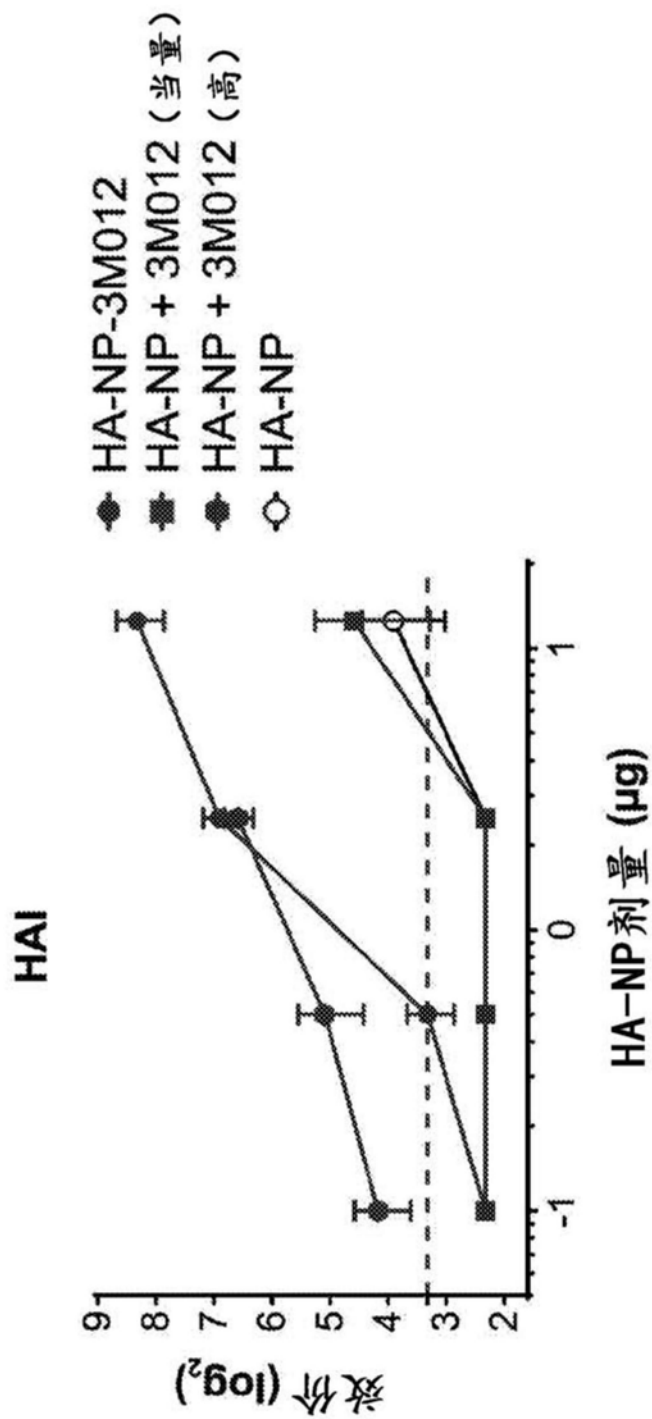


图26B