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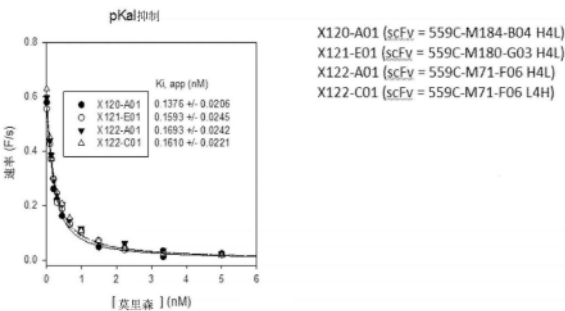
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(54) 发明名称

针对血浆激肽释放酶和因子XII的双特异性抗体

(57) 摘要

本发明的发明名称为针对血浆激肽释放酶和因子XII的双特异性抗体。本文描述的是结合血浆激肽释放酶 (pK1) 和因子XII的双特异性抗体以及生产这类双特异性抗体的方法和使用这类双特异性抗体治疗与接触系统相关的疾病或病症 (例如遗传性血管性水肿和血栓形成) 的方法。本公开的一个方面是双特异性抗体, 其包含第一多肽和第二多肽, 所述第一多肽包含第一抗体的轻链, 所述轻链包含轻链可变区 (V_L) 和轻链恒定区 (C_L) (例如 κ 轻链或 λ 轻链); 所述第二多肽包含第一抗体的重链, 所述重链包含重链可变区 (V_H) 和重链恒定区 (C_H)。



1. 一种双特异性抗体,其包含:

第一多肽,其包含第一抗体的轻链,所述轻链包含轻链可变区(V_L)和轻链恒定区(C_L);
和

第二多肽,其包含第一抗体的重链,所述重链包含重链可变区(V_H)和重链恒定区(C_H),
其中所述第一多肽或所述第二多肽进一步包含第二抗体,所述第二抗体是包含重链可变区(V_H)和轻链可变区(V_L)的单链抗体,并且融合至所述第一多肽或所述第二多肽的C-末端;和

其中所述第一抗体结合血浆激肽释放酶(pKa1),并且包括HYIMM所示的重链互补决定区(HCDR)1,GIYSSGGITVYADSVKG所示的HCDR2,和RRIGVPRRDEFDI所示的HCDR3;以及RASQSISSWLA所示的轻链互补决定区(LCDR)1,KASTLES所示的LCDR2,和QQYNTYWT所示的LCDR3;和

其中所述第二抗体结合活性因子XII(FXIIa)并且包括:

(i) SEQ ID NO:41所示的HCDR1,SEQ ID NO:43所示的HCDR2,和SEQ ID NO:45所示的HCDR3;以及SEQ ID NO:34所示的LCDR1,SEQ ID NO:36所示的LCDR2,和SEQ ID NO:40所示的LCDR3;

(ii) SEQ ID NO:42所示的HCDR1,SEQ ID NO:44所示的HCDR2,和SEQ ID NO:45所示的HCDR3;以及SEQ ID NO:34所示的LCDR1,SEQ ID NO:36所示的LCDR2,和SEQ ID NO:37所示的LCDR3;

(iii) SEQ ID NO:135所示的HCDR1,SEQ ID NO:136所示的HCDR2,和SEQ ID NO:134所示的HCDR3;以及SEQ ID NO:131所示的LCDR1,SEQ ID NO:36所示的LCDR2,和SEQ ID NO:37所示的LCDR3;

(iv) SEQ ID NO:137所示的HCDR1,SEQ ID NO:138所示的HCDR2,和SEQ ID NO:134所示的HCDR3;以及SEQ ID NO:131所示的LCDR1,SEQ ID NO:36所示的LCDR2,和SEQ ID NO:37所示的LCDR3;或

(v) SEQ ID NO:139所示的HCDR1,SEQ ID NO:140所示的HCDR2,和SEQ ID NO:134所示的HCDR3;以及SEQ ID NO:131所示的LCDR1,SEQ ID NO:36所示的LCDR2,和SEQ ID NO:37所示的LCDR3。

2. 根据权利要求1所述的双特异性抗体,其中所述第二抗体融合至所述第二多肽的C-末端。

3. 根据权利要求1或2所述的双特异性抗体,其中所述第一抗体是IgG。

4. 根据权利要求3所述的双特异性抗体,其中所述IgG包含突变的重链,所述突变的重链与野生型对应物相比C-末端赖氨酸残基被缺失或突变。

5. 根据权利要求4所述的双特异性抗体,其中所述第一抗体的所述重链包含C-末端甘氨酸残基,而不是赖氨酸残基。

6. 根据权利要求1或2所述的双特异性抗体,其中所述第二抗体在其C-末端不包含KR基序。

7. 根据权利要求1或2所述的双特异性抗体,其中所述双特异性抗体是四价的。

8. 根据权利要求1或2所述的双特异性抗体,其中所述第二多肽包含在所述第一抗体的所述重链和所述第二抗体之间的肽连接体。

9. 根据权利要求8所述的双特异性抗体,其中所述肽连接体是SGGGS。
10. 根据权利要求1或2所述的双特异性抗体,其中所述C_L是κ轻链。
11. 根据权利要求1或2所述的双特异性抗体,其中所述C_L是λ轻链。
12. 根据权利要求1或2所述的双特异性抗体,其中在所述第二抗体中,所述V_H融合至所述V_L的N-末端。
13. 根据权利要求1或2所述的双特异性抗体,其中在所述第二抗体中,所述V_H融合至所述V_L的C-末端。
14. 根据权利要求9所述的双特异性抗体,其中所述第二抗体包含在所述V_H区和所述V_L区之间的肽连接体。
15. 根据权利要求14所述的双特异性抗体,其中所述肽连接体是(G₄S)₄。
16. 根据权利要求1或2所述的双特异性抗体,其中在所述第二抗体中的所述V_H链和所述V_L链之间形成二硫键。
17. 根据权利要求16所述的双特异性抗体,其中所述二硫键在V_H链中位置44处的半胱氨酸和V_L链中位置100处的半胱氨酸之间形成。
18. 根据权利要求1或2所述的双特异性抗体,其中所述第一抗体的V_H包含SEQ ID NO:1的氨基酸序列。
19. 根据权利要求1或2所述的双特异性抗体,其中所述第一抗体的所述重链包含SEQ ID NO:9的位置20至位置470的氨基酸残基。
20. 根据权利要求19所述的双特异性抗体,其中所述第一抗体的所述重链包含SEQ ID NO:9、149或150的氨基酸序列。
21. 根据权利要求1或2所述的双特异性抗体,其中所述第一抗体的所述V_L包含SEQ ID NO:2的氨基酸序列。
22. 根据权利要求1或2所述的双特异性抗体,其中所述第二抗体的所述V_H包含SEQ ID NO:3、4和124-126的氨基酸序列中的任一种。
23. 根据权利要求1或2所述的双特异性抗体,其中所述第二抗体的所述V_L包含来自SEQ ID NO:5-8和127的氨基酸序列中任一个的位置1至位置111的氨基酸残基。
24. 根据权利要求1或2所述的双特异性抗体,其中所述第二抗体的所述V_L包含SEQ ID NO:5-8和127的氨基酸序列中的任一种。
25. 根据权利要求1或2所述的双特异性抗体,其中所述第一多肽包含SEQ ID NO:10的氨基酸序列和所述第二多肽包含选自SEQ ID NO:11、17、48-50、75-76和143-148组成的组的氨基酸序列。
26. 一种双特异性抗体,其包含结合至血浆激肽释放酶(pKa1)的第一抗体和结合至活性因子XIIa(FXIIa)的第二抗体,
其中第一抗体包括HYIMM所示的重链互补决定区(HCDR)1,GIYSSGGITVYADSVKG所示的HCDR2,和RRIGVPRRDEFDI所示的HCDR3;以及RASQSISSWLA所示的轻链互补决定区(LCDR)1, KASTLES所示的LCDR2,和QQYNTYWT所示的LCDR3;和
其中第二抗体包括包含以下的重链可变区(VH)和轻链可变区(VL):
(i) SEQ ID NO:41所示的HCDR1,SEQ ID NO:43所示的HCDR2,和SEQ ID NO:45所示的HCDR3;以及SEQ ID NO:34所示的LCDR1,SEQ ID NO:36所示的LCDR2,和SEQ ID NO:40所示

的LCDR3;

(ii) SEQ ID NO:42所示的HCDR1, SEQ ID NO:44所示的HCDR2, 和SEQ ID NO:45所示的HCDR3; 以及SEQ ID NO:34所示的LCDR1, SEQ ID NO:36所示的LCDR2, 和SEQ ID NO:37所示的LCDR3;

(iii) SEQ ID NO:135所示的HCDR1, SEQ ID NO:136所示的HCDR2, 和SEQ ID NO:134所示的HCDR3; 以及SEQ ID NO:131所示的LCDR1, SEQ ID NO:36所示的LCDR2, 和SEQ ID NO:37所示的LCDR3;

(iv) SEQ ID NO:137所示的HCDR1, SEQ ID NO:138所示的HCDR2, 和SEQ ID NO:134所示的HCDR3; 以及SEQ ID NO:131所示的LCDR1, SEQ ID NO:36所示的LCDR2, 和SEQ ID NO:37所示的LCDR3; 或

(v) SEQ ID NO:139所示的HCDR1, SEQ ID NO:140所示的HCDR2, 和SEQ ID NO:134所示的HCDR3; 以及SEQ ID NO:131所示的LCDR1, SEQ ID NO:36所示的LCDR2, 和SEQ ID NO:37所示的LCDR3。

27. 根据权利要求26所述的双特异性抗体, 其中所述第一抗体的所述 V_H 包含SEQ ID NO:1的氨基酸序列, 和/或所述第一抗体的所述 V_L 包含SEQ ID NO:2的氨基酸序列。

28. 根据权利要求26或27所述的双特异性抗体, 其中所述第二抗体的所述 V_H 链包含SEQ ID NO:3、4和124-126的氨基酸序列中的任一种; 和/或所述第二抗体的所述 V_L 包含SEQ ID NO:5-8和127的氨基酸序列中的任一个的残基1-111。

29. 分离的核酸或核酸组, 其包含编码根据权利要求1-28中任一项所述的第一多肽的第一核苷酸序列和编码根据权利要求1-28中任一项所述的第二多肽的第二核苷酸序列。

30. 根据权利要求29所述的核酸或核酸组, 其中所述第一核苷酸序列和所述第二核苷酸序列位于两个分开的核酸分子上。

31. 根据权利要求29所述的核酸或核酸组, 其中所述第一核酸核苷酸序列和所述第二核酸核苷酸序列位于一个核酸分子上。

32. 根据权利要求30所述的核酸或核酸组, 其为包含第一载体和第二载体的载体组, 所述第一载体包含第一核苷酸序列, 所述第二载体包含第二核苷酸序列。

33. 根据权利要求32所述的核酸或核酸组, 其中所述第一载体和所述第二载体是表达载体, 在所述表达载体中, 所述第一核苷酸序列和所述第二核苷酸序列各自可操作地与启动子连接。

34. 根据权利要求31所述的核酸或核酸组, 其为包含所述第一核苷酸序列和所述第二核苷酸序列两者的载体。

35. 根据权利要求34所述的核酸或核酸组, 其中所述载体是表达载体, 在所述表达载体中, 所述第一核苷酸序列和所述第二核苷酸序列可操作地与共同的启动子或不同的启动子连接。

36. 一种药物组合物, 其包含 (i) 根据权利要求1-28中任一项所述的双特异性抗体或根据权利要求29-35中任一项所述的核酸或核酸组, 和 (ii) 药学上可接受的载体。

37. 宿主细胞或宿主细胞组, 其包含根据权利要求29-35中任一项所述的核酸或核酸组, 其中所述宿主细胞或宿主细胞组为细菌细胞、酵母细胞、昆虫细胞或哺乳动物细胞。

38. 用于制备双特异性抗体的方法, 所述方法包括:

在允许第一多肽和第二多肽表达的条件下培养根据权利要求37所述的宿主细胞或宿主细胞组;和

分离包含所述第一多肽和所述第二多肽的所述双特异性抗体。

39.根据权利要求38所述的方法,其中所述宿主细胞包含表达载体,所述表达载体包含编码所述第一多肽的第一核苷酸序列和编码所述第二多肽的第二核苷酸序列。

40.根据权利要求1-28中任一项所述的双特异性抗体或权利要求29-35中任一项所述的核酸或核酸组在制备用于治疗与接触激活系统相关的疾病的药物中的用途,其中所述与接触激活系统相关的疾病是遗传性血管性水肿或血栓形成。

41.一种试剂盒,其包括:(i)根据权利要求1-28中任一项所述的双特异性抗体或权利要求29-35中任一项所述的核酸或核酸组或权利要求36所述的药物组合物,和(ii)向受试者施用(i)的所述双特异性抗体、核酸或核酸组或药物组合物的说明书。

针对血浆激肽释放酶和因子XII的双特异性抗体

[0001] 本申请为申请日是2015年12月31日、申请号是201580075633.1 (PCT/US2015/068238)、发明名称为“针对血浆激肽释放酶和因子XII的双特异性抗体”的中国申请的分案申请。

[0002] 相关申请的交叉引用

[0003] 本申请要求美国临时申请第62/099,236号(2015年1月2日提交)、美国临时申请第62/200,363号(2015年8月3日提交)和美国临时申请第62/261,609号(2015年12月1日提交)的申请日的权益。这些引用的申请的全部内容通过引用并入本文。

发明背景

[0004] 因子XII (FXII) 是将前激肽释放酶转变成血浆激肽释放酶 (pKa1) 的主要激活因子。激活的血浆激肽释放酶裂解(切割, cleave) 高分子量激肽原 (HMWK), 以释放缓激肽 (BK)。PKa1也能将潜伏因子XII激活成为活性因子XII (因子XIIa)。在与接触系统的异常激活相关的疾病状态(诸如遗传性血管性水肿)中, BK的不受控制的水平可引起患者疾病发作。

发明概述

[0005] 本公开的一个方面是双特异性抗体, 其包含: 第一多肽和第二多肽, 所述第一多肽包含第一抗体的轻链, 所述轻链包含轻链可变区 (V_L) 和轻链恒定区 (C_L) (例如 κ 轻链或 λ 轻链), 所述第二多肽包含第一抗体的重链, 所述重链包含重链可变区 (V_H) 和重链恒定区 (C_H)。所述双特异性抗体的第一多肽或第二多肽进一步包含第二抗体, 所述第二抗体为单链抗体并且可被融合至第一多肽或第二多肽的C-末端。第一抗体和第二抗体中的一个结合血浆激肽释放酶 (pKa1) (例如活性pKa1) 并且另外一个抗体结合因子XII (例如活性因子XII或FXIIa), 例如, 第一抗体结合pKa1且第二抗体结合FXIIa或反之亦然。

[0006] 在一些实施方案中, 第一抗体是IgG。在一个实施例中, IgG包含突变的重链, 所述突变的重链与野生型对应物相比, C-末端赖氨酸残基被缺失或突变。例如, 第一抗体突变的重链可包含C-末端甘氨酸残基, 而不是如在野生型IgG重链中的赖氨酸残基。在一个实施例中, 双特异性抗体可以是四价的。

[0007] 在一些实施方案中, 在双特异性抗体中的第二多肽包含第一抗体的重链和第二抗体之间的肽连接体。在一个实施例中, 肽连接体可为SGGGS (SEQ ID NO:22)。

[0008] 在第二抗体(其为scFv抗体)中, V_H 可被融合至 V_L 的N-末端。可替代地, V_H 被融合至 V_L 的C-末端。在一些实施例中, 第二抗体包含 V_H 区和 V_L 区之间的肽连接体, 例如(G_4S)₄的连接体 (SEQ ID NO:23)。在一些实施方案中, scFv抗体包含在 V_H 链和 V_L 链之间形成的二硫键。例如, V_H 链可包含在位置44 (C44) 处的半胱氨酸残基, 和 V_L 链可包含在位置100处的半胱氨酸残基, 其中二硫键可在 V_H 中的 C_{44} 和 V_L 中的 C_{100} 之间形成。在一些实施例中, 第二抗体在其C-末端不包含KR基序。

[0009] 在本文描述的任何双特异性抗体中, 第一抗体的 V_H 具有与SEQ ID NO:1中的互补

决定区 (CDR) 相同的互补决定区 (CDR)。在一些实施例中, 第一抗体的 V_H 包含SEQ ID NO:1的氨基酸序列。在一个实施例中, 第一抗体的重链包含SEQ ID NO:9的残基20-470的氨基酸序列。在一个实施例中, 第一抗体的重链包含SEQ ID NO:9、149和150的氨基酸序列。可替代地或另外, 第一抗体的 V_L 具有与SEQ ID NO:2中的CDR相同的CDR。在一些实施例中, 第一抗体的 V_L 包含SEQ ID NO:2的氨基酸序列。

[0010] 进一步地, 第二抗体的 V_H 可具有与SEQ ID NO:3、4和123-126中的任一个的CDR相同的CDR。在一些实施例中, 第二抗体的 V_H 包含SEQ ID NO:3、4和123-126的氨基酸序列中的任一种。可替代地或另外, 第二抗体的 V_L 具有与SEQ ID NO:5-8和127-130的任一个中的CDR相同的CDR。在一些实施例中, 第二抗体的 V_L 包含SEQ ID NO:5-8和127的氨基酸序列中的任一个的残基1-111。在一个实施例中, 第二抗体的 V_L 包含SEQ ID NO:5-8和127-130的氨基酸序列中的任一种。

[0011] 在一些实施例中, 本文所述的的双特异性抗体包含第一多肽和第二多肽, 所述第一多肽包含SEQ ID NO:10的氨基酸序列, 所述第二多肽包含SEQ ID NO:11-20、47-122、141-148和151-158的氨基酸序列中的任一种。

[0012] 在另一个方面中, 本公开提供了双特异性抗体, 其包含结合至血浆激肽释放酶 (pKal) 的第一抗体和结合至因子XII的第二抗体, 例如结合至活性pKal的第一抗体和/或结合至活性因子XII (FXIIa) 的第二抗体。在一些实施方案中, 第一抗体包含 V_H 链和/或 V_L 链, 所述 V_H 链包含与SEQ ID NO:1中的互补决定区 (CDR) 相同的互补决定区 (CDR), 所述 V_L 链包含与SEQ ID NO:2中的CDR相同的CDR。例如, 第一抗体的 V_H 包含SEQ ID NO:1的氨基酸序列, 和/或第一抗体的 V_L 包含SEQ ID NO:2的氨基酸序列。

[0013] 可替代地或另外, 第二抗体包含 V_H 链和/或 V_L 链, 所述 V_H 链包含与SEQ ID NO:3或4中的CDR相同的CDR, 所述 V_L 链包含与SEQ ID NO:5、6、7或8中的CDR相同的CDR。例如, 第二抗体的 V_H 链包含SEQ ID NO:3或4的氨基酸序列; 和/或第二抗体的 V_L 包含SEQ ID NO:5、6、7或8的氨基酸序列。

[0014] 可替代地或另外, 第二抗体包含 V_H 链和/或 V_L 链, 所述 V_H 链包含与SEQ ID NO:123-126任一个中的CDR相同的CDR, 所述 V_L 链包含与SEQ ID NO:127中的CDR相同的CDR。例如, 第二抗体的 V_H 链包含SEQ ID NO:123-126的任一氨基酸序列; 和/或第二抗体的 V_L 包含SEQ ID NO:5-8和127的氨基酸序列中的任一个的残基1-111。

[0015] 在另一方面中, 本公开提供了分离的核酸或核酸组, 其包含编码本文所述的第一多肽或第一抗体的第一核苷酸序列和编码本文所述的第二多肽或第二抗体的第二核苷酸序列。在一些实施方案中, 第一核苷酸序列和第二核苷酸序列位于两个分开的核酸分子上 (例如两个载体, 诸如表达载体)。可替代地, 第一核酸核苷酸序列和第二核酸核苷酸序列位于一个核酸分子上 (例如载体, 诸如表达载体)。

[0016] 本文所述的核酸或核酸组可以是包含第一载体和第二载体的载体组, 所述第一载体包含第一核苷酸序列, 所述第二载体包含第二核苷酸序列。在一些实施例中, 第一载体和第二载体是表达载体, 其中, 第一核苷酸序列和第二核苷酸序列可操作地与启动子连接。在其他实施例中, 本文所述的核酸是包含第一核苷酸序列和第二核苷酸序列两者的载体。本文所述的任何载体可以是表达载体。例如, 表达载体可包含第一核苷酸序列和第二核苷酸序列, 其可操作地与启动子连接。包含本文所述的载体或载体组的宿主细胞也在本公开的

范围内。

[0017] 进一步地,本公开提供了包含本文所述的任何双特异性抗体或核酸/核酸组和药学上可接受的载体的组合物。这类组合物可用于治疗与接触激活系统相关的疾病(例如遗传性血管性水肿(HAE)或血栓形成)。本文所述的治疗方法包括向有需要的受试者施用有效量的本文所述的药物组合物。本公开也提供了用于治疗本文所述疾病的药物组合物,其中所述药物组合物包含本文所述的任何双特异性抗体或编码所述双特异性抗体的核酸/核酸组,和药学上可接受的载体,以及提供了这类药物组合物在制备用于治疗诸如HAE或血栓形成的这类疾病的药物中的用途。在一些实施方案中,血栓形成与心房纤维颤动、深部静脉血栓形成(DVT)、肺栓塞、中风或动脉或静脉血栓形成事件相关。

[0018] 在又一方面中,本公开的特征在于制备双特异性抗体的方法,所述方法包括:(a)在允许第一多肽和第二多肽表达的条件下培养本文所述的宿主细胞或宿主细胞组;和(b)分离包含第一多肽和第二多肽的双特异性抗体。在一些实施例中,宿主细胞包含表达载体,所述表达载体包含编码第一多肽的第一核苷酸序列和编码第二多肽的第二核苷酸序列。

[0019] 在下面的说明中阐释了本公开的一个或多个实施方案的细节。通过以下附图和对几个实施方案的详细说明,以及通过附加的权利要求书,本公开的其他特征或优点将是显而易见的。

附图简述

[0020] 以下附图组成本说明书的一部分,并且被包括以进一步证明本公开的某些方面,通过参考这些附图中的一个或多个结合对本文所示的具体实施方案的详细说明,可以更好地理解本公开。

[0021] 图1是显示各种双特异性抗体克隆抑制pKa1的活性的图,所述克隆包括克隆X120-A01(scFv=559C-M184-B04 H4L)、X121-E01(scFv=559C-M184-G03 H4L)、X122-A01(scFv=559C-M71-F06 H4L)和X122-C01(scFv=559C-M71-F06 L4H)。

[0022] 图2包括的图显示克隆X120-A01(A和B)、X122-A01(C)、X121-E01(D)、X122-C01(E)和对照克隆M71-F06 IgG(F)的FXIIa抑制活性。

[0023] 图3包括的图显示5种示例性双特异性分子的分析尺寸排阻色谱(SEC)踪迹。前面的峰显示这些克隆具有高分子量聚集体,范围是%HMW 16.5-33.8。A:620I-X136-C11。B:620I-X136-C05。C:620I-X136-G05。D:620I-X136-D12。E:620I-X136-A01。

[0024] 图4包括的图显示在一系列浓度下,620I-X0173-A11(具有H44/L100工程化的二硫键的620I-X0136-D12)的高分子量聚集体的减少。A:620I-X0173-A11,在1mg/mL。B:620I-X0173-A11,在10mg/mL。C:620I-X0173-A11,在20mg/mL。D:620I-X0173-A11,在45mg/mL。

[0025] 图5包括的图显示如通过重构血浆测定(reconstituted plasma assay)所测定的抗pKa1抗体、抗FXIIa抗体、抗pKa1抗体和抗FXIIa抗体的组合和双特异性抗体D12的抑制活性。A:DX-2930,在单链HMWK存在的情况下。B:抗FXIIa抗体,在单链HMWK存在的情况下。C:DX-2930+抗FXIIa,在单链HMWK存在的情况下。D:双特异性克隆620I-X0136-D12,在单链HMWK存在的情况下。

[0026] 图6包括的图显示如通过重构血浆测定所测定的抗pKa1抗体、抗FXIIa抗体、抗pKa1抗体和抗FXIIa抗体的组合和双特异性抗体D12的抑制活性。A:DX-2930,在不存在HMWK

的情况下。B:抗FXIIa抗体,在不存在HMWK的情况下。C:DX-2930+抗FXIIa,在不存在HMWK的情况下。D:双特异性克隆620I-X0136-D12,在不存在HMWK的情况下。

[0027] 图7的图显示在活化部分凝血活酶时间 (APTT) 测定中与抗FXIIa抗体 (D06) 和抗pKa1抗体 (H03) 相比,双特异性抗体 (D12) 在三种浓度的效果。

[0028] 图8为显示双特异性抗体候选物620I-X0136-D12 (D12) 抑制纤维蛋白形成的能力的图。

[0029] 图9包括的图显示620I-X0173-A11 (具有二硫化物的620I-X0136-D12) 针对pKa1 (上面的传感图) 和FXIIa (下面的传感图) 的biacore结合。A:pKa1结合 (上面的曲线) 较空白表面 (中间) 和前激肽释放酶 (下部) 高。最初的FXIIa分离株 (isolate) 显示对biacore芯片非特异性的结合,这解释了针对前Ka1和空白看到的结合信号。B:FXIIa结合 (上面的曲线) 明显高于FXII (下部) 和空白 (中间)。

[0030] 图10包括的图显示 IC_{50} 和 K_i ,在血浆抑制测定 (Plasma Inhibition Assay) 中3种二硫化物约束的 (disulfide-constrained) 双特异性抗体的表观计算结果。A和B:克隆620I-X0173-A11。C和D:克隆620I-X0173-C07。E和F:克隆620I-X0173-G11。

[0031] 图11的图显示在血浆抑制测定中所测定的双特异性抗体620I-X0177-A01 (也称为620I-X0173-A11) 的抑制特征。

[0032] 图12包括的图显示在亲本IgG和双特异性抗体之间亲和力的急下降。A:亲本克隆559C-X0211-A01 (左图) 和双特异性抗体620I-X0177-A01 (右图) 的结合特征。B:亲本克隆DX-2930 (左图) 和双特异性抗体A01 (右图) 的结合特征。

[0033] 图13的图表显示通过所述各种抗体,APTT的剂量依赖性延迟。克隆D06、1A01和F12是抗FXIIa抗体。克隆H03是抗pKa1抗体。克隆D12和7A01是分别不具有二硫键和具有二硫键的双特异性抗体。

[0034] 图14的图表显示通过克隆1A01 (559C-X211-A01) 和7A01 (620I-X0177-A01),纤维蛋白沉积的剂量依赖性延迟。

[0035] 图15是SDS-PAGE蛋白凝胶,其显示在还原条件下 (泳道2-4) 和非还原条件下 (泳道6-8) 双特异性抗体620I-X0177-A01的样本。

[0036] 图16包括的图显示双特异性抗体620I-X0177-A01的分析尺寸排阻色谱 (SEC) 踪迹,展示pH依赖性裂解。在15.7-16.1分钟之间的峰表示正确形成的双特异性抗体。在17分钟的峰表示DX-2930IgG1。在22分钟的峰表示裂解的单链抗体。A:pH 6.0。B:pH 7.0。C:8.0。

[0037] 图17包括在 $t=0$ 时被工程化为突变或者缺失IgG C-末端赖氨酸的指定的双特异性抗体的SDS-PAGE蛋白凝胶。A:非还原条件。B:还原条件。

[0038] 图18显示SDS-PAGE蛋白凝胶,其包括在还原条件下于 $t=48$ 小时被工程化为突变或者缺失IgG C-末端赖氨酸的指定的双特异性抗体。阳性对照是双特异性抗体620I-X0177-A01。

[0039] 图19包括的图显示对照双特异性抗体620I-X0177-A01的分析尺寸排阻色谱 (SEC) 踪迹。在15.7-16.1分钟之间的峰表示正确形成的双特异性抗体。在17分钟的峰表示DX-2930IgG1。在22分钟的峰表示裂解的单链抗体。A: $t=0$ 。B: $t=48$ 小时。

[0040] 图20包括的图显示再工程化的双特异性抗体在室温, $pH=7.5$, 48小时后的分析尺寸排阻色谱 (SEC) 踪迹。A:620I-X180-E07。B:620I-X180-G03。C:620I-X180-A05。D:620I-

X180-E06.E:620I-X180-C11.F:620I-X179-C01.G:620I-X179-G05.H:620I-X179-A09。

[0041] 图21显示SDS-PAGE蛋白凝胶,其显示在非还原条件下被工程化为突变或缺失重链IgG C-末端赖氨酸的指定的双特异性抗体。阳性对照是620I-X0177-A01。样本用内蛋白酶Lys C在37℃孵育1小时。

[0042] 图22包括的图显示实例双特异性抗体的pKa1抑制。板(plate)1显示双特异性抗体620I-X0179-A09(空心圆)、620I-X0179-C01(实心三角形)和620I-X0179-E05(空心三角形)的抑制特征。板2显示双特异性抗体620I-X0179-G05(空心圆)、620I-X0180-E07(实心三角形)和620I-X0180-G03(空心三角形)的抑制特征。板3显示双特异性抗体620I-X0180-A05(空心圆)和620I-X0180-C11(实心三角形)的抑制特征。在每一个板中,抗体DX-2930被用作对照。

[0043] 图23包括的图显示实例双特异性抗体的FXIIa抑制。板1显示双特异性抗体620I-X0179-A09(空心圆)、620I-X0179-C01(实心三角形)和620I-X0179-E05(空心三角形)的抑制特征。板2显示双特异性抗体620I-X0179-G05(空心圆)、620I-X0180-E07(实心三角形)和620I-X0180-G03(空心三角形)的抑制特征。板3显示双特异性抗体620I-X0180-A05(空心圆)和620I-X0180-C11(实心三角形)的抑制特征。在每个板中抗体DX-4012(559C-M0192-H11)被用作对照。

[0044] 图24包括的图显示实例双特异性抗体对活化血浆的抑制。左上图显示来自对照板1、2和3的DX-2930和DX-4012的抑制特征。右上图显示双特异性抗体620I-X0179-A09(实心圆)、620I-X0179-C01(空心圆)的抑制特征。左下图显示双特异性抗体620I-X0179-E05(实心圆)、620I-X0179-G05(空心圆)和620I-X0180-E07(实心三角形)的抑制特征。右下图显示双特异性抗体620I-X0180-G03(实心圆)、620I-X0180-A05(空心圆)和620I-X0180-C11(实心三角形)的抑制特征。

发明详述

[0045] 接触激活系统通过促炎性肽缓激肽(BK)的释放而发起内源性凝血途径。BK释放通过接触激活系统中的一系列酶激活步骤而得到促进。因子XIIa(FXIIa)将前激肽释放酶转变成血浆激肽释放酶(pKa1)。然后激活的pKa1裂解高分子量激肽原(HMWK)以释放缓激肽(BK)。重要的是,pKa1也可激活潜伏的因子XII以产生额外的活性因子XIIa。认为形成了正反馈环,其中pKa1将FXII激活成FXIIa,和FXIIa将前激肽释放酶激活成pKa1。

[0046] 在与接触激活系统相关的疾病(诸如遗传性血管性水肿(HAE)和血栓形成)中,BK的不受控制的水平可引起炎症反应,诸如患者HAE发作。因此,用于控制BK水平的药剂,例如pKa1和FXII的抑制剂,可具有重要的治疗价值。

[0047] 本文所述的是结合pKa1和FXII(例如活性pKa1和/或FXIIa)两者的双特异性抗体,以及其在抑制pKa1和FXII两者中的用途和治疗与接触激活系统相关的疾病(诸如遗传性血管性水肿(HAE)和血栓形成)中的用途。如在以下实施例中所示,如本文所述的许多示例性双特异性抗体经证明抑制pKa1和FXIIa两者的活性。不希望受理论的限制,预期本文所述的双特异性抗体与可抑制pKa1或FXII的药剂相比,在治疗与接触激活系统相关的疾病中表现出优越的治疗效果,因为双特异性抗体能够抑制pKa1和FXII两者的活性,从而通过例如阻断pKa1和FXII之间的正反馈环而协调地降低BK水平。

[0048] 结合至pKa1和FXII的双特异性抗体

[0049] 如本文所使用的,抗体(可互换地使用复数形式)是免疫球蛋白分子,或其功能性片段,其能够通过位于免疫球蛋白分子的可变区内的至少一个抗原识别位点而结合靶抗原,诸如碳水化合物、多核苷酸、脂质或多肽。多特异性抗体,例如双特异性抗体,是免疫球蛋白分子或其功能性片段/变体,其能够结合多个靶抗原,例如两个抗原或一个抗原上的两个表位。本文所述的双特异性抗体可结合至血浆激肽释放酶(pKa1)和因子XII两者。在一些实施方案中,双特异性抗体可结合和抑制活性pKa1和FXIIa两者。

[0050] 如本文所使用的,抗原指具有产生抗体的能力的任何分子(例如蛋白质、核酸、多糖或脂质)。表位是抗体所结合的抗原的部分(例如pKa1或FXII的部分)。表位通常由部分(诸如氨基酸或多糖侧链)的化学活性(诸如极性、非极性或疏水性)表面基团(grouping)组成,并且可具有特异性三维结构特点,同样具有特异性电荷特点。表位本质上可以是线性的或可以是不连续的表位,例如构象表位,所述构象表位是通过抗原的非连续的氨基酸之间的空间关系形成的,而不是通过线性氨基酸系列形成的。构象表位包括由抗原的折叠而产生的表位,其中来自抗原的线性序列的不同部分的氨基酸在3维空间中极为接近。

[0051] 本文所述的双特异性抗体包含两个抗体部分,结合至pKa1(例如活性pKa1)的第一抗体部分和结合至FXII(例如FXIIa)的第二抗体部分。第一抗体部分和第二抗体部分可来源于能够结合至期望的抗原(即pKa1(例如活性pKa1)和FXII(例如FXIIa))的两个亲本抗体。用于构建本文所述的双特异性抗体的亲本抗体中的一个或两个可以是天然存在的抗体(例如来源于合适的供体,诸如人、小鼠、大鼠、兔、马或羊的抗体)、遗传工程化的抗体(例如人源化抗体、嵌合抗体)或来源于天然抗体文库或合成抗体文库的抗体。在一些实施方案中,一个亲本抗体是IgG抗体,例如结合至pKa1的IgG抗体(诸如DX-2930)或结合至FXIIa的IgG抗体,而另一个亲本抗体是scFv抗体,例如结合至FXIIa的scFv抗体(诸如本文所述的抗FXIIa克隆)或结合至pKa1的scFv抗体。

[0052] 天然存在的IgG分子的重链通常在其C-末端含有赖氨酸残基。在一些实施方案中,该C-末端赖氨酸残基可以被缺失或突变,例如在本文公开的双特异性抗体中被突变为甘氨酸残基。可替代地或另外,通常存在于轻链可变区和轻链恒定区的接合处的KR基序在本文所述的双特异性抗体中可以从第一抗体、第二抗体或两者的轻链删除。在一些实施例, KR基序从本文所述的任何双特异性分子的scFv部分(例如在scFv的C-末端处)删除。这些突变可降低双特异性抗体的蛋白水解裂解和/或改善双特异性抗体的表达、生产和/或制造。

[0053] 在一些实施例,至少一个亲本抗体可以是亲和力成熟的抗体,其指的是这样的抗体,所述抗体与未经修饰的亲本抗体相比,在一个或多个CDR或框架区(FR)中具有一个或多个修饰,导致抗体对靶抗原亲和力的改善。优选的亲和力成熟的抗体对靶抗原可具有纳摩尔或甚至是皮摩尔的亲和力。可通过本领域已知的各种方法进行抗体的亲和力成熟,所述方法包括可变结构域重排(shuffling)(参见例如Marks等,1992,Bio/Technology 10:779-783)、CDR和/或FR残基的随机诱变(见例如Barbas等,1994,Proc Nat. Acad. Sci, USA 91:3809-3813; Schier等,1995, Gene 169:147-155; Yelton等,1995, J. Immunol. 155:1994-2004; Jackson等,1995, J. Immunol. 154(7):3310-9和Hawkins等,1992, J. Mol. Biol. 226:889-896)。亲本抗体可以是任何类别(诸如IgD、IgE、IgG、IgA或IgM)或其亚类别或单链抗体,诸如scFv。

[0054] 本文所述的双特异性抗体中的每个抗体部分可以是以任何形式的抗体,包括但不限于完整的(即全长)抗体、其抗原结合片段(诸如Fab、Fab'、F(ab')₂、Fv)、单链抗体(scFv抗体)和四价抗体。在一些实施方案中,双特异性抗体是四价的,其包含针对pKa1的两个结合位点和针对FXII的两个结合位点。

[0055] 在一些实施方案中,本文所述的双特异性抗体中的抗pKa1部分、抗FXII部分或两者特异性地结合至相应的靶抗原或其表位。“特异性地结合”至抗原或表位的抗体是本领域中悉知的术语,并且确定这类特异性结合的方法在本领域也是熟知的。如果一个分子相比可替代的靶标更加频繁、更加迅速、以更长的持续时间和/或以更高的亲和力与特定靶抗原反应或缔合,则认为这个分子展示“特异性结合”。与抗体与其他物质的结合相比,如果抗体以更高的亲和力、亲合力,更加容易和/或以更长的持续时间结合靶抗原或表位,则该抗体“特异性结合”至该靶抗原或表位。例如,特异性地(或优先地)结合至抗原(例如人pKa1或FXII)或其中的抗原表位的抗体是这样的抗体,所述抗体相比其与其它抗原或相同抗原内的其他表位的结合,与该靶抗原以更高的亲和力、亲合力、更容易和/或以更长的持续时间进行结合。通过阅读该定义也可理解,例如,特异性结合至第一靶抗原的抗体可以特异性地或优先地结合至第二靶抗原或可以不特异性地或优先地结合至第二靶抗原。同样地,“特异性结合”或“优先结合”并非一定要求(尽管其可以包括)专一性结合。通常(但不一定),提及结合意为优先结合。在一些实施例中,“特异性地结合”至靶抗原或其表位的抗体可以不结合至其它抗原或相同抗原中的其他表位。在一些实施方案中,本文所述的双特异性抗体特异性地结合至活性pKa1和FXIIa两者。

[0056] 在一些实施方案中,本文所述的双特异性抗体对于靶抗原(例如pKa1或FXIIa)中的一种或两者或其抗原表位具有合适的结合亲和力。如本文所用,“结合亲和力”指的是表观缔合常数或 K_A 。 K_A 是解离常数(K_D)的倒数。对于靶抗原中的一种或两者或抗原表位,本文所述的双特异性抗体可具有至少 10^{-5} 、 10^{-6} 、 10^{-7} 、 10^{-8} 、 10^{-9} 、 10^{-10} M或更低的结合亲和力(K_D)。增加的结合亲和力对应于减少的 K_D 。相对于第三抗原,抗体对第一抗原和第二抗原较高的亲和力结合,可以通过相比针对结合第三抗原的 K_A (或数值 K_D)针对结合第一抗原和第二抗原的较高的 K_A (或更小的数值 K_D)来指示。在这类情况中,相对于第三抗原(例如处于第二构象的相同的第一或第二蛋白质或其模拟物;或第三蛋白质),抗体对第一抗原和第二抗原(例如处于第一构象的第一蛋白质或其模拟物和处于第一构象的第二蛋白质或其模拟物)具有特异性。结合亲和力的差异(例如对于特异性或其他比较)可至少为1.5、2、3、4、5、10、15、20、37.5、50、70、80、91、100、500、1000、10,000或 10^5 倍。

[0057] 结合亲和力(或结合特异性)可通过各种方法确定,所述方法包括平衡透析、平衡结合、凝胶过滤、ELISA、表面等离子体共振或光谱法(例如使用荧光测定)。用于评估结合亲和力的示例性条件是在HBS-P缓冲液(10mM HEPES pH7.4、150mM NaCl、0.005% (v/v) 表面活性剂P20)中。这些技术可用于测量随着靶蛋白浓度而变化的结合的结合蛋白的浓度。结合的结合蛋白的浓度([结合的])与游离靶蛋白的浓度([游离的])和靶上的结合蛋白的结合位点的浓度相关,其中,根据下述方程,(N)是每个靶分子的结合位点的数目:

[0058]
$$[\text{结合的}] = [N][\text{游离的}] / (K_d + [\text{游离的}])$$

[0059] 但是,并不总是有必要对 K_A 进行精确测定,因为有时足以获得对亲和力的定量测量,例如使用比如ELISA或FACS分析所测定的,其与 K_A 成比例,因此可用于比较,比如确定较

高的亲和力是否是例如2倍,以获得对亲和力的定性测量,或获得对亲和力的推导,例如通过功能测定例如体外测定或体内测定中的活性。

[0060] (i) 抗pKa1部分

[0061] 任何能够结合至pKa1 (诸如活性pKa1) 的抗体可用于构建本文所述的双特异性抗体。在一些实施例中,双特异性抗体中的抗pKa1抗体部分可结合至人pKa1,并抑制其活性至少50% (例如60%、70%、80%、90%、95%或更高)。抑制常数(K_i) 提供对抑制剂效力的度量;其是将酶活性降低一半所需的抑制剂浓度,不依赖于酶或底物浓度。本文所述的双特异性抗体中的抗pKa1抗体部分的抑制活性可由常规方法测定。在一些实施例中,本文所述的双特异性抗体具有低于1nM (例如0.5nM、0.2nM、0.1nM、0.09nM、0.08nM、0.07nM、0.06nM、0.05nM、0.04nM、0.03nM、0.02nM、0.01nM或更低) 的抗pKa1 $K_{i,app}$ 值。抗体的 $K_{i,app}$ 值可遵循本领域已知的方法估计。

[0062] 在一些实施方案中,双特异性抗体的抗pKa1部分可与以下残基中的一个或多个相互作用:人pKa1中的V410、L412、T413、A414、Q415、R416、L418、C419、H434、C435、F436、D437、G438、L439、W445、Y475、K476、V477、S478、E479、G480、D483、F524、E527、K528、Y552、D554、Y555、A564、D572、A573、C574、K575、G576、S578、T596、S597、W598、G599、E600、G601、C602、A603、R604、Q607、P608、G609、V610和Y611。包含涉及的氨基酸残基 (以粗体和下划线显示) 的人pKa1的C-末端片段的氨基酸序列显示如下 (SEQ ID NO:21):

391-IVGGTNSSWG EWPWQVSLQV KLTAQRHLCG GSLIGHQWVL TAAHCFDGLP
 LQDVWRIYSG ILNLSDITKD TPF~~SQ~~IK~~EII~~ IHQNYKVSEG NHDIALIKLQ
 [0063] APLNYTEFQK PISLPSKGDT STIYTNCWVT GWGFSKEEKGE IQNILQKVNI
 PLVTNEECQK RYQDYKITQR MVCAGYKEGG KDACKGDSGG PLVCKHNGMW
 RLVGITTSWGE GCARREQPGV YTKVAEYMDW ILEKTQSSDG KAQM~~Q~~SPA -638

[0064] 在一些实施例中,抗pKa1抗体部分可结合pKa1的表位,所述表位包括以上所示的SEQ ID NO:21中的以下片段中的一个:V410-C419、H434-L439、Y475-G480、F524-K528、Y552-Y555、D572-S578、T596-R604或Q607-Y611。

[0065] 在一个实施例中,本文所述的双特异性抗体的抗pKa1部分来源于抗体DX-2930,所述抗体DX-2930在US 20120201756 (通过引用并入本文) 中被描述。DX-2930的重链可变区和轻链可变区,以及该抗体的全长重链和轻链提供如下 (CDR区:以粗体和下划线显示;信号序列:以斜体显示):

[0066] DX-2930的重链可变区 (SEQ ID NO:1):

EVQLLES~~GGGLVQ~~PGGSLRLS~~CAASGFTFS~~HYIMMWVRQAPGKGLEWVSGIYSSGGI
 [0067] TVYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQ
 GTMVTVSS

[0068] DX-2930的轻链可变区 (SEQ ID NO:2):

DIQMTQSPSTLSASVGD~~RV~~TITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESG
 [0069] VPSRFGSGSGTEFTLTIS~~SLQ~~PDDFATYYCQYNTYWTFGQGTKVEIK

[0070] DX-2930重链 (SEQ ID NO:9):

[0071] MGWSCIIILFLVATATGAHSEVQLLES~~GGGLVQ~~PGGSLRLS~~CAASGFTFS~~HYIMMWVRQAPGKGLEWVSG
 IYSSGGITVYADSVKGRFTISRDN~~SKNTLYL~~QMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG

PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSHE
ALHNHYTQKSLSLSPGK

[0072] DX-2930轻链 (SEQ ID NO:10) :

[0073] MGWSCIIILFLVATATGVHSDIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYK
ASTLESGVPSRFSGSGSGTEFTLTISSLQPDDFATYYCQQYNTYWTFGGQTKVEIKRTVAAPSVFIFPPSDEQLKSG
TASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPV
TKSFNRGEC

[0074] 在一些实施例中,双特异性抗体的抗pKa1部分包含重链可变区和/或轻链可变区,所述重链可变区包含与SEQ ID NO:1具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列,所述轻链可变区包含与SEQ ID NO:2具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列。两个氨基酸序列的“同一性百分比”利用Karlin和Altschul Proc.Natl.Acad.Sci.USA 87:2264-68,1990的算法 (在Karlin和Altschul Proc.Natl.Acad.Sci.USA 90:5873-77,1993中被改良) 测定。这种算法被并入到Altschul,等.J.Mol.Biol.215:403-10,1990的NBLAST和XBLAST程序 (2.0版本) 中。可用XBLAST程序,得分=50,字长=3进行BLAST蛋白质搜寻,以获得与目标蛋白质分子同源的氨基酸序列。在两个序列之间存在缺口的情况下,可以利用如Altschul等,Nucleic Acids Res.25 (17): 3389-3402,1997中所述的Gapped BLAST。当利用BLAST和Gapped BLAST程序时,可以利用各个程序 (例如XBLAST和NBLAST) 的默认参数。

[0075] 在其他实施例中,本文所述的双特异性抗体中的抗pKa1部分包含重链可变区,所述重链可变区包含与SEQ ID NO:1中的那些CDR相同的三个CDR和/或与SEQ ID NO:2中的那些CDR相同的三个CDR。具有相同CDR的两个重链可变区 (或两个轻链可变区) 意味着在这两个重链可变区 (或轻链可变区) 中通过相同的编码方案所确定的CDR是相同的。用于确定抗体CDR的示例性编码方案包括“Kabat”编码方案 (Kabat等 (1991), 第五版, Public Health Service, National Institutes of Health, Bethesda, Md.)、“Chothia”编码方案 (Al-Lazikani等, (1997) JMB 273, 927-948)、“Contact”编码方案 (MacCallum等, J.Mol.Biol.262:732-745 (1996))、“IMGT”编码方案 (Lefranc M P等, Dev Comp Immunol, 2003 January; 27 (1): 55-77) 和“Aho”编码方案 (Honegger A和Pluckthun A, J Mol Biol, 2001 Jun. 8; 309 (3): 657-70)。如本领域技术人员所知,本文所鉴定的示例性抗pKa1和抗FXII抗体的CDR区通过“Chothia”编码方案 (作为实例) 确定。

[0076] 可替代地,相比SEQ ID NO:1和/或SEQ ID NO:2,抗pKa1部分在一个或多个CDR中可包括一个或多个 (例如多达2、3、4、5、6、7或8个) 突变。这类突变可以是保守的氨基酸取代。如本文所用,“保守的氨基酸取代”指的是不改变产生氨基酸取代的蛋白质的相对电荷或尺寸特性的氨基酸取代。氨基酸的保守取代包括在以下组中的氨基酸中进行的取代: (a) M、I、L、V; (b) F、Y、W; (c) K、R、H; (d) A、G; (e) S、T; (f) Q、N; 和 (g) E、D。

[0077] 在本文所述的任何实施例中,相比参考抗体,双特异性抗体的抗pKa1部分可包含一个或多个 (例如1、2、3、4、5或更多个) 突变或缺失。可引入这类突变,例如以降低双特异性

抗体的蛋白水解裂解,和/或改善双特异性抗体的表达、生产和/或制造。在一些实施方案中,双特异性抗体的抗pKa1部分是IgG,并且与其野生型对应物相比,IgG的重链的C-末端赖氨酸残基被去除或突变。在一些实施方案中,IgG重链C-末端赖氨酸被突变成中性氨基酸残基,例如甘氨酸残基或丙氨酸残基。

[0078] 双特异性抗体的抗pKa1部分的这类突变的重链的示例性序列提供如下(使用DX-2930的重链作为实例)。

[0079] C-末端赖氨酸残基缺失的DX-2930重链(SEQ ID NO:149)

[0080] MGWSCIIILFLVATATGAHSEVQLLESGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSMHE
ALHNHYTQKSLSLSPG

[0081] C-末端赖氨酸突变为甘氨酸的DX-2930重链(SEQ ID NO:150)

[0082] MGWSCIIILFLVATATGAHSEVQLLESGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFN
WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP
SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVMSMHE
ALHNHYTQKSLSLSPGG

[0083] 上文提供的序列的斜体部分指的是信号肽。本文公开的双特异性抗体的抗pKa1部分可包含相同的信号肽,可以信号肽被去除或被不同的信号肽代替。用于生产分泌蛋白质的信号肽在本领域是熟知的。

[0084] 双特异性抗体中的抗pKa1部分可以是任何抗体形式,包括但不限于完整的(即全长)抗体、其抗原结合片段(诸如Fab、Fab'、F(ab')₂、Fv)和单链抗体。在一些实施例中,本文所述的抗pKa1部分的重链可变区与重链恒定区(C_H)连接,所述重链恒定区可以是重链恒定区的全长或其部分(即C_H1、C_H2、C_H3或其组合)。重链恒定区可来源于本领域已知的任何C_H。在一些实施方案中,C_H是γ重链。可替代地或另外,抗pKa1部分的轻链可变区与轻链恒定区(C_L)连接,所述轻链恒定区可以是本领域已知的任何C_L。在一些实施例中,C_L是κ轻链。在其他实施例中,C_L是λ轻链。抗体重链和轻链恒定区在本领域是熟知的,例如在IMGT数据库(www.imgt.org)或在www.vbase2.org/vbstat.php中提供的那些重链和轻链恒定区,其都通过引用并入本文。在一些实施例中,抗pKa1部分是IgG,其可包含与DX-2930(SEQ ID NO:9)相同的重链和/或与DX-2930(SEQ ID NO:10)相同的轻链。

[0085] 可替代地,本文所述的双特异性抗体中的抗pKa1部分可以是单链抗体(ScFv),在所述单链抗体中,重链可变区和轻链可变区例如通过肽连接体诸如(GGGGS)₄的连接体(SEQ ID NO:23)而被融合。在一个实施例中,重链可变区和轻链可变区在H→L方向上融合。在其他实施例中,重链可变区和轻链可变区在L→H方向上融合。在一些实施方案中,ScFv的轻链

部分在其C-末端不包含Lys-Arg(KR)基序。

[0086] 在一个实施例中,本文所述的双特异性抗体中的抗pKa1部分是本文所述的DX-2930(IgG抗体),其包含SEQ ID NO:9的重链和SEQ ID NO:10的轻链或其抗原结合片段。

[0087] (ii) 抗FXII部分

[0088] 任何能够结合至FXII,诸如活性FXII(FXIIa),的抗体均可用于构建本文所述的双特异性抗体。在一些实施例中,双特异性抗体中的抗FXII抗体部分可结合至人FXIIa并抑制其至少50%(例如60%、70%、80%、90%、95%或更高)的活性。本文所述的双特异性抗体中的抗FXII抗体部分的抑制活性可由常规的方法测定。在一些实施例中,本文所述的双特异性抗体具有低于1nM(例如0.5nM、0.2nM、0.1nM、0.09nM、0.08nM、0.07nM、0.06nM、0.05nM、0.04nM、0.03nM、0.02nM、0.01nM或更低)的抗FXIIa $K_{i,app}$ 值。抗体的 $K_{i,app}$ 值可遵循本领域已知的方法估计。

[0089] 在一些实施例中,本文所述的双特异性抗体的抗FXII部分来源于抗FXII克隆559C-M0071-F06、559C-M0179-D04、559C-M0181-C02、559C-M0180-G03和559C-M0184-B04。这些克隆的重链可变区和轻链可变区提供如下(CDR以粗体和下划线显示):

[0090] 克隆559C-M0071-F06、559C-M0179-D04、559C-M0181-C02和559C-M0180-G03的重链可变区(SEQ ID NO:3):

[0091] EVQLLES GGGLVQPGGSLRLSCAASGFTFS **GYIMAWVRQAPGKGLEWVS** **YIYPSGGI**
TVYADSVKGRRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTR **QRYRGPKY**YYMDVWG
KGT TVTVSS

[0092] 克隆559C-M0184-B04的重链可变区(SEQ ID NO:4):

[0093] EVQLLES GGGLVQPGGSLRLSCAASGFTFS **FYSMH**WVRQAPGKGLEWVS **RIYPSGGV**
TKYADSVKGRRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTR **QRYRGPKY**YYMDVWG
KGT TVTVSS

[0094] 克隆559C-M0071-F06和559C-M0184-B04的轻链可变区(SEQ ID NO:5):

[0095] DIQMTQSPLSLPVTPGEPASISCR **RSSQSLLHSNGYNYLD**WYLQKPGQSPQLLIY **LGS**
NRASGVVPDRFSGSGSGTDFTLTKISRVEAEDVGVYYC **MQALQTPWT**FGQGTKVEIKR

[0096] 克隆559C-M0179-D04的轻链可变区(SEQ ID NO:6):

[0097] DIQMTQSPLSLSVAPGEPASISCR **RSSQSLLHRNGHNYLD**WYLQKPGQSPQLLIY **LGS**
NRASGVPERFSGSGSGTDFTLRISRVEAEDVGVYYC **MQALQART**FGQGTKVEIKR

[0098] 克隆559C-M0181-C02的轻链可变区(SEQ ID NO:7):

[0099] DIQMTQSPLSLPVTPGEPASISCR **RSSQSLLHSNGYNYLD**WYLQKPGQSPQLLIY **LGS**
NRASGVVPDRFSGSGSGTDFTLTKISRVEAEDVGVYYC **MQALQTRT**FGQGTKVEIKR

[0100] 克隆559C-M0180-G03的轻链可变区(SEQ ID NO:8):

[0101] DIQMTQSPLSLPVTPGEPASISCR **RSSQSLLHSNGYNYLD**WYLQKPGQSPQIMIIY **LGS**
NRASGVVPDRFSGSGSGTDFTLTKISRVEAEDVGVYYC **MQALQTPRT**FGQGTKVEIKR

[0102] 克隆620I-X0173-A11(620I-X0177-A01)的重链可变区(SEQ ID NO:123)

[0103] EVQLLES GGGLVQPGGSLRLSCAASGFTFS **QYVMH**WVRQAPGKCLEWVS **SIWPSGGH**
TRYADSVKGRRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR **QRYRGPKY**YYMDVWG
QGT TVTVSS

- [0104] 克隆620I-X0173-C07 (620I-X0177-C01)的重链可变区 (SEQ ID NO:124)
EVQLLES GGGLVQPGGSLRLSCAASGFTFS WYVMHWVRQAPGKCLEWVS SIYPSGGK
- [0105] TSYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAR QRYRGPKYYYMDVWG
QGTTVTVSS
- [0106] 克隆620I-X0173-E07 (620I-X0177-E01)的重链可变区 (SEQ ID NO:125)
EVQLLES GGGLVQPGGSLRLSCAASGFTFS WYSMHWVRQAPGKCLEWVS VIYPSGGK
- [0107] TRYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAR QRYRGPKYYYMDVWG
QGTTVTVSS
- [0108] 克隆620I-X0173-G11 (620I-X0177-G01)的重链可变区 (SEQ ID NO:126)
EVQLLES GGGLVQPGGSLRLSCAASGFTFS HYVMHWVRQAPGKCLEWVS SIYPSGGL
- [0109] TKYADSVKGRFTISRDN SKNTLYLQMN SLRAEDTAVYYCAR QRYRGPKYYYMDVWG
QGTTVTVSS
- [0110] 克隆620I-X0173-A11的轻链可变区 (SEQ ID NO:127)
DIVMTQSPLSLPVTPGEPASISCR SSQSLLHSNGYNYLDWYLQKPGQSPQLLIY LGS
- [0111] NRASGV PDRFSGSGSGTDFTLKI SRVEAEDVG VYYC MQALQTPWTFGCGTKVEIKR
- [0112] 克隆620I-X0173-C07的轻链可变区 (SEQ ID NO:128)
DIVMTQSPLSLPVTPGEPASISCR SSQSLLHSNGYNYLDWYLQKPGQSPQLLIY LGS
- [0113] NRASGV PDRFSGSGSGTDFTLKI SRVEAEDVG VYYC MQALQTPWTFGCGTKVEIKR
- [0114] 克隆620I-X0173-E07的轻链可变区 (SEQ ID NO:129)
DIVMTQSPLSLPVTPGEPASISCR SSQSLLHSNGYNYLDWYLQKPGQSPQLLIY LGS
- [0115] NRASGV PDRFSGSGSGTDFTLKI SRVEAEDVG VYYC MQALQTPWTFGCGTKVEIKR
- [0116] 克隆620I-X0173-G11的轻链可变区 (SEQ ID NO:130)
DIVMTQSPLSLPVTPGEPASISCR SSQSLLHSNGYNYLDWYLQKPGQSPQLLIY LGS
- [0117] NRASGV PDRFSGSGSGTDFTLKI SRVEAEDVG VYYC MQALQTPWTFGCGTKVEIKR

[0118] 克隆620I-X0173-A11 (SEQ ID No:127)、620I-X0173-C07 (SEQ ID NO:128)、620I-X0173-E07 (SEQ ID NO:129)和620I-X0173-G11 (SEQ ID NO:130)的轻链可变区是相同的。

[0119] 在一些实施例中,双特异性抗体的抗FXIIa部分包含重链可变区和/或轻链可变区,所述重链可变区包含与SEQ ID NO:3或SEQ ID NO:4具有至少80% (例如85%、90%、95%或98%)同一性的氨基酸序列,所述轻链可变区包含与SEQ ID NO:5-8任一个具有至少80% (例如85%、90%、95%或98%)同一性的氨基酸序列。例如,重链可变区可包含与SEQ ID NO:3具有至少80% (例如85%、90%、95%或98%)同一性的氨基酸序列和轻链可变区可包含与SEQ ID NO:5-8任一个具有至少80% (例如85%、90%、95%或98%)同一性的氨基酸序列。可替代地,重链可变区可包含与SEQ ID NO:4具有至少80% (例如85%、90%、95%或98%)同一性的氨基酸序列和轻链可变区可包含与SEQ ID NO:5具有至少80% (例如85%、90%、95%或98%)同一性的氨基酸序列。

[0120] 在一些实施例中,双特异性抗体的抗FXIIa部分包含重链可变区和/或轻链可变区,所述重链可变区包含与SEQ ID NO:123-126任一个具有至少80% (例如85%、90%、95%或98%)同一性的氨基酸序列,所述轻链可变区包含与SEQ ID NO:127-130任一个具有至少80% (例如85%、90%、95%或98%)同一性的氨基酸序列。例如,重链可变区可包含与SEQ

ID NO:123具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列和轻链可变区可包含与SEQ ID NO:127具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列。可替代地,重链可变区可包含与SEQ ID NO:124具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列和轻链可变区可包含与SEQ ID NO:128具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列。可替代地,重链可变区可包含与SEQ ID NO:125具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列和轻链可变区可包含与SEQ ID NO:129具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列。可替代地,重链可变区可包含与SEQ ID NO:126具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列和轻链可变区可包含与SEQ ID NO:130具有至少80% (例如85%、90%、95%或98%) 同一性的氨基酸序列。

[0121] 在其他实施例中,本文所述的双特异性抗体中的抗FXIIa部分包含重链可变区和/或轻链可变区,其包含如克隆559C-M0071-F06、559C-M0179-D04、559C-M0181-C02、559C-M0180-G03、559C-M0184-B04、620I-X0173-A11、620I-X0173-C07、620I-X0173-E07或620I-X0173-G11中那些CDR相同的三个CDR,和/或如这些克隆中的那些CDR相同的三个CDR。见如下表1:

[0122] 表1:抗FXIIa克隆的CDR序列:

克隆	LCDR1	LCDR2	LCDR3	HCDR1	HCDR2	HCDR3	
[0123]	559C-M 0071-F0 6	RSSQSLLHS NGY NYLD (SEQ ID NO:34)	LGSNRAS (SEQ ID NO:36)	MQALQTP WT (SEQ ID NO:37)	GYIMA (SEQ ID NO:41)	YIYPSGGITV YA DSVKG (SEQ ID NO:43)	QRYRGPKYYY YYMDV (SEQ ID NO:45)
	559C-M 0179-D0 4	RSSQSLLHR NGH NYLD (SEQ ID NO:35)	LGSNRAS (SEQ ID NO:36)	MQALQAR T (SEQ ID NO:38)	GYIMA (SEQ ID NO:41)	YIYPSGGITV YA DSVKG (SEQ ID NO:43)	QRYRGPKYYY YYMDV (SEQ ID NO:45)
	559C-M 0181-C0 2	RSSQSLLHS NGY NYLD (SEQ ID NO:34)	LGSNRAS (SEQ ID NO:36)	MQALQTR T (SEQ ID NO:39)	GYIMA (SEQ ID NO:41)	YIYPSGGITV YA DSVKG (SEQ ID NO:43)	QRYRGPKYYY YYMDV (SEQ ID NO:45)
	559C-M 0180-G0 3	RSSQSLLHS NGY NYLD (SEQ ID NO:34)	LGSNRAS (SEQ ID NO:36)	MQALQTP RT (SEQ ID NO:40)	GYIMA (SEQ ID NO:41)	YIYPSGGITV YA DSVKG (SEQ ID NO:43)	QRYRGPKYYY YYMDV (SEQ ID NO:45)
	559C-M 0184-B0 4	RSSQSLLHS NGY NYLD (SEQ ID NO:34)	LGSNRAS (SEQ ID NO:36)	MQALQTP WT (SEQ ID NO:37)	FYSMH (SEQ ID NO:42)	RIYPSGGVT KYA DSVKG (SEQ ID NO:44)	QRYRGPKYYY YYMDV (SEQ ID NO:45)
	620I-X0 173-A11	RSSQSLLHS NGYNYLD (SEQ ID NO: 131)	LGSNRAS (SEQ ID NO: 36)	MQALQTP WT (SEQ ID NO:37)	QYVMH (SEQ ID NO: 132)	SIWPSGGHT RYADSVKG (SEQ ID NO: 133)	QRYRGPKYYY YMDV (SEQ ID NO: 134)
	620I-X0 173-C07	RSSQSLLHS NGYNYLD (SEQ ID NO: 131)	LGSNRAS (SEQ ID NO: 36)	MQALQTP WT (SEQ ID NO:37)	WYVMH (SEQ ID NO: 135)	SIYPSGGKTS YADSVKG (SEQ ID NO: 136)	QRYRGPKYYY YMDV (SEQ ID NO: 134)
620I-X0	RSSQSLLHS	LGSNRAS	MQALQTP	WYSMH	VIYPSGGKT	QRYRGPKYYY	
[0124]	173-E07	NGYNYLD (SEQ ID NO: 131)	(SEQ ID NO: 36)	WT (SEQ ID NO:37)	(SEQ ID NO: 137)	RYADSVKG (SEQ ID NO: 138)	YMDV (SEQ ID NO: 134)
	620I-X0 173-G11	RSSQSLLHS NGYNYLD (SEQ ID NO: 131)	LGSNRAS (SEQ ID NO: 36)	MQALQTP WT (SEQ ID NO:37)	HYVMH (SEQ ID NO: 139)	SIYPSGGLTK YADSVKG (SEQ ID NO: 140)	QRYRGPKYYY YMDV (SEQ ID NO: 134)

[0125] 可替代地,相比克隆559C-M0071-F06、559C-M0179-D04、559C-M0181-C02、559C-M0180-G03、559C-M0184-B04、620I-X0173-A11、620I-X0173-C07、620I-X0173-E07或620I-X0173-G11中的任一个,抗FXIIa部分在上文表1中所列出的一个或多个重链和/或轻链CDR中可包括一个或多个(例如多达2、3、4、5、6、7或8个)突变。这类突变可以是本文所述的保守的氨基酸取代。

[0126] 在一些实施方案中,本文所述的双特异性抗体中的抗FXIIa部分是IgG分子,其可以是天然存在的IgG或突变体,例如包含一个或多个(例如1、2、3、4、5或更多个)突变或缺失,例如以降低双特异性抗体的蛋白水解裂解、降低双特异性抗体的电荷异质性、和/或改善双特异性抗体的表达、生产和/或制造。在一些实施方案中,与其野生型对应物相比,IgG的重链的C-末端赖氨酸残基被去除或突变。在一些实施方案中,IgG重链C-末端赖氨酸被突

变为中性氨基酸残基,例如甘氨酸残基或丙氨酸残基。

[0127] 双特异性抗体中的抗FXIIa部分可以是任何抗体形式,包括但不限于完整的(即全长)抗体、其抗原结合片段(诸如Fab、Fab'、F(ab')₂、Fv)和单链抗体。在一些实施例中,本文所述的抗pKa1部分的重链可变区与重链恒定区(C_H)连接,所述重链恒定区可以是重链恒定区的全长或其部分(例如C_H1、C_H2、C_H3或其组合)。重链恒定区可来源于本领域已知的任何C_H。在一些实施方案中,C_H是γ重链。可替代地或另外,抗pKa1部分的轻链可变区与轻链恒定区(C_L)连接,所述轻链恒定区可以是本领域已知的任何C_L。在一些实施例中,C_L是κ轻链。在其他实施例中,C_L是λ轻链。抗体重链和轻链恒定区在本领域内是熟知的,例如本文所述的。

[0128] 可替代地,本文所述的双特异性抗体中的抗FXIIa部分可以是单链抗体,在所述单链抗体中,重链可变区和轻链可变区例如通过柔性(flexible)肽连接体诸如(GGGGS)₄的连接体(SEQ ID NO:23)被融合。重链可变区和轻链可变区可在H→L方向上融合或可在L→H方向上融合。在一些实施方案中,ScFv的轻链部分在其C-末端不包含KR基序。

[0129] 在一些实施方案中,抗FXIIa部分是包含SEQ ID NO:3或SEQ ID NO:4的重链可变区和/或SEQ ID NO:5-8任一个的轻链可变区的scFv。在一个实施例中,抗FXIIa部分是在H→L或L→H方向上包含SEQ ID NO:3的重链可变区和SEQ ID NO:5-8任一个的轻链可变区的scFv抗体。在其他实施例中,抗FXIIa部分是在H→L或L→H方向上包含SEQ ID NO:4的重链可变区和SEQ ID NO:5的轻链可变区的scFv抗体。在一些实施方案中,抗FXIIa部分是包含SEQ ID NO:123-126任一个的重链可变区和/或SEQ ID NO:127-130任一个的轻链可变区的scFv。在一个实施例中,抗FXIIa部分是在H→L或L→H方向上包含SEQ ID NO:123的重链可变区和SEQ ID NO:127的轻链可变区的scFv抗体。在一个实施例中,抗FXIIa部分是在H→L或L→H方向上包含SEQ ID NO:123的重链可变区和SEQ ID NO:127的轻链可变区的scFv抗体。在另一个实施例中,抗FXIIa部分是在H→L或L→H方向上包含SEQ ID NO:124的重链可变区和SEQ ID NO:128的轻链可变区的scFv抗体。在另一个实施例中,抗FXIIa部分是在H→L或L→H方向上包含SEQ ID NO:125的重链可变区和SEQ ID NO:129的轻链可变区的scFv抗体。在另一个实施例中,抗FXIIa部分是在H→L或L→H方向上包含SEQ ID NO:126的重链可变区和SEQ ID NO:130的轻链可变区的scFv抗体。

[0130] 在一些实施方案中,本文所述的任何scFv抗体的重链可变区和轻链可变区例如通过诸如V_H残基44和V_L100残基之间的二硫键被进一步连接。

[0131] (iii) 抗pKa1/抗FXII双特异性抗体的形式

[0132] 本文所述的抗pKa1/抗FXIIa双特异性抗体可以为本领域已知的任何双特异性抗体形式,例如在Klein等,mAbs 4(6):653-663,2012;Kontermann等,mAbs 4(2):182-197,2012;和Coloma等,Nature Biotechnology 15:159-163,1997中所述的那些双特异性抗体形式。在一些实施例中,双特异性抗体可以是杂交全长抗体(也称为四价体瘤(quadroma)或三官能抗体),其包含与pKa1结合的一个臂(重链/轻链复合体)和与FXII结合的另一个臂(重链/轻链复合体)。在一些实施例中,双特异性抗体是双特异性Fab'₂或三-Fab分子,所述双特异性Fab'₂包含与pKa1结合的一个Fab片段和与FXII结合的另一个Fab片段,所述三-Fab分子包含与一个靶抗原(例如pKa1或FXIIa)结合的Fab片段的两个拷贝和与另一靶抗原(例如FXIIa或pKa1)结合的Fab片段的一个拷贝。可替代地,双特异性抗体是串联的scFv分子,其包含与pKa1结合的scFv的至少一个拷贝和与FXIIa结合的另一个scFv的一个拷贝。本

文所述的双特异性抗体也可以是本领域已知的双抗体或单链双抗体。其他实例包括但不限于IgG₂、F(ab')₂、CovX-体、scFv₄-Ig、IgG-scFv、scFv-IgG、DVD-Ig、IgG-sVD、sVD-IgG、二合一型IgG(2-in-1-IgG)、mAb²、串联抗体共同(Tandemab common)LC、kih IgG、kih IgG共同LC、CrossMab、kih IgG-scFab、mAb-Fv、电荷对、SEED-体、双抗体(Db)、dsDd、scDb、tandAbs、串联scFv、三联抗体(triple body)、Fab-scFv和F(ab')₂-scFv₂。见,例如,Kontermann等,mAbs4(2):182-197,2012的图2。

[0133] 在一些实施方案中,本文所述的双特异性抗体的支架被设计成包含IgG抗体部分和scFv部分,所述scFv部分被融合至IgG部分的重链或轻链的C-末端(例如IgG的重链的C-末端,见例如Coloma,M.J.&Morrison,S.L.Design and production of novel tetravalent bispecific antibodies.Nature Biotechnology.15(2):159-163.1997)。IgG重链或轻链可通过短肽连接体诸如富含甘氨酸残基和丝氨酸残基的肽与scFv融合。在一个实施例中,肽连接体包含SGGGS的氨基酸序列(SEQ ID NO:22)。

[0134] 这类双特异性抗体可包含第一多肽和第二多肽,所述第一多肽包含第一抗体的轻链,所述轻链包含轻链可变区(V_L)和轻链恒定区(C_L);所述第二多肽包含融合蛋白,其从N-末端至C-末端包含第一抗体的重链,所述重链包含重链可变区(V_H)、重链恒定区(C_H)和第二抗体,所述第二抗体可以是单链抗体。可替代地,双特异性抗体可包含第一多肽和第二多肽,所述第一多肽包含第一抗体的重链,所述重链包含重链可变区(V_H)和重链恒定区(C_H)或其部分;所述第二多肽包含融合蛋白,其从N-末端至C-末端包含第一抗体的轻链和第二抗体,所述轻链包含轻链可变区(V_L)和轻链恒定区(C_L),所述第二抗体是单链抗体。在一些实施例中,第一抗体可结合至pKa1(例如活性pKa1)和第二抗体可结合至FXII(例如FXIIa)。在其他实施例中,第一抗体可结合至FXIIa和第二抗体可结合至pKa1。

[0135] 第一抗体的轻链的C_L可以是本领域中已知的任何C_L。在一些实施方案中,C_L是κ轻链。在一些实施方案中,C_L是λ轻链。第一抗体的重链的C_H可以是本领域中已知的任何C_H。在一些实施方案中,C_H是γ重链。这类重链和轻链恒定区在本领域中是熟知的,例如,如本文所述。

[0136] 在一个实施例中,双特异性抗体在H→L或L→H方向上包括来源于DX-2930的IgG抗体和来源于克隆559C-M0071-F06、559C-M0179-D04、559C-M0181-C02、559C-M0180-G03、559C-M0184-B04、620I-X0173-A11、620I-X0173-C07、620I-X0173-E07或620I-X0173-G11的scFv抗体。见以上公开内容。来源于亲本抗体的抗体可包含与亲本抗体的重链和轻链基本上类似(共享至少80%、85%、90%、95%或98%的序列同一性)的重链和轻链。在一些实施例中,这类抗体包含与亲本抗体相同的重链和轻链CDR。在其他实施例中,这类抗体包含与亲本抗体的CDR基本上一致的重链和/或轻链CDR,例如与亲本抗体的CDR相比包含多达5、4、3、2或1个氨基酸残基变异,诸如保守的氨基酸残基取代。

[0137] 在一些实施方案中,双特异性抗体中的scFv抗体包含融合至V_L的N-末端的V_H。在其他实施方案中,scFv抗体包含融合至V_L的C-末端的V_H。在本文所述的任何scFv抗体中,V_H区和V_L区可以通过连接体(诸如肽连接体)融合。

[0138] 本文所述的肽连接体可包含例如1、2、3、4、5、6、7、8、9、10或更多氨基酸残基。在一些实施方案中,肽连接体可包含2-50、5-25或5-20个氨基酸。在一些实施方案中,肽连接体是SGGGS。在一些实施方案中,肽连接体是(G₄S)_x,其中x可以是1、2、3、4、5、6、7、8、9、10或更

大。在一些实施方案中,x是4。

[0139] 本文所述的任何肽连接体,例如SGGGs (SEQ ID NO:22) 连接体或(GGGGS)₄ (SEQ ID NO:23) 连接体,可包含天然存在的氨基酸和/或非天然存在的氨基酸。天然存在的氨基酸包括丙氨酸(Ala)、精氨酸(Arg)、天冬酰胺(Asn)、天门冬氨酸(Asp)、半胱氨酸(Cys)、谷氨酸(Glu)、谷氨酰胺(Gln)、甘氨酸(Gly)、组氨酸(His)、异亮氨酸(Ile)、亮氨酸(Leu)、赖氨酸(Lys)、蛋氨酸(Met)、鸟氨酸(Orn)、苯丙氨酸(Phe)、脯氨酸(Pro)、丝氨酸(Ser)、苏氨酸(Thr)、色氨酸(Trp)、酪氨酸(Tyr)和缬氨酸(Val)。非天然存在的氨基酸可包括受保护的氨基酸,诸如用基团诸如乙酰基、甲酰基、甲苯磺酰基、硝基等保护的天然存在的氨基酸。非天然存在的氨基酸的非限制性实例包括叠氮基高丙氨酸、高炔丙基甘氨酸、高烯丙基甘氨酸、对-溴苯丙氨酸、对-碘苯丙氨酸、叠氮基苯丙氨酸、乙酰基苯丙氨酸或乙炔基苯丙氨酸、包含内烯烃(诸如反式巴豆基烯烃)的氨基酸、丝氨酸烯丙基醚、烯丙基甘氨酸、炔丙基甘氨酸、乙烯基甘氨酸、吡咯赖氨酸、N-σ-邻-叠氮基苄氧基羰基-L-赖氨酸(AzZLys)、N-σ-炔丙氧基羰基-L-赖氨酸、N-σ-2-叠氮基乙氧基羰基-L-赖氨酸、N-σ-叔-丁氧基羰基-L-赖氨酸(BocLys)、N-σ-烯丙氧基羰基-L-赖氨酸(AlocLys)、N-σ-乙酰基-L-赖氨酸(AcLys)、N-σ-苄氧基羰基-L-赖氨酸(ZLys)、N-σ-环戊氧基羰基-L-赖氨酸(CycLys)、N-σ-D-脯氨酰基-L-赖氨酸、N-σ-烟酰基-L-赖氨酸(NicLys)、N-σ-N-Me-氨茴酰-L-赖氨酸(NmaLys)、N-σ-生物素基-L-赖氨酸、N-σ-9-苄基甲氧基羰基-L-赖氨酸、N-σ-甲基-L-赖氨酸、N-σ-二甲基-L-赖氨酸、N-σ-三甲基-L-赖氨酸、N-σ-异丙基-L-赖氨酸、N-σ-丹酰-L-赖氨酸、N-σ-邻,对-二硝基苯基-L-赖氨酸、N-σ-对-甲苯磺酰基-L-赖氨酸、N-σ-DL-2-氨基-2-羧乙基-L-赖氨酸、N-σ-苯基丙酮酰胺-L-赖氨酸、N-σ-丙酮酰胺-L-赖氨酸、叠氮基高丙氨酸、高炔丙基甘氨酸、高烯丙基甘氨酸、对-溴苯丙氨酸、对-碘苯丙氨酸、叠氮基苯丙氨酸、乙酰基苯丙氨酸或乙炔基苯丙氨酸、包含内烯烃(诸如反式巴豆基烯烃)的氨基酸、丝氨酸烯丙基醚、烯丙基甘氨酸、炔丙基甘氨酸和乙烯基甘氨酸。

[0140] 在一些实施方案中,本文所述的双特异性抗体的scFv部分可被工程化,以在V_H链和V_L链两者中引入半胱氨酸残基,用以形成一个或多个二硫键,其可以降低高分子量聚集体的形成。在一些实施例中,半胱氨酸残基可被引入到V_H链的残基44中。可替代地或另外,半胱氨酸残基可被引入到V_L链的残基100中。

[0141] 示例性抗pKa1/抗FXIIa双特异性抗体包括在以下实施例中所述的克隆X0120-A01、X0120-C01、X0120-E01、X0120-G01、X0121-A03、X0121-C01、X0121-E01、X0121-G01、X0122-A01、X0122-C01、620I-X0173-A11、620I-X0173-C07、620I-X0173-E07和620I-X0173-G11。其它示例性抗pKa1/抗FXIIa双特异性抗体包括在以下实施例中所述的克隆620I-X138-A08、620I-X136-B02、620I-X139-A12、620I-X137-B08、620I-X142-A04、620I-X142-B11、620I-X138-B01、620I-X136-C01、620I-X138-A12、620I-X136-A12、620I-X138-A02、620I-X136-A05、620I-X138-C07、620I-X136-E07、620I-X142-B02、620I-X136-F11、620I-X142-A05、620I-X136-C09、620I-X138-B10、620I-X136-C08、620I-X139-A11、620I-X136-D05、620I-X138-D04、620I-X136-G08、620I-X142-B07、620I-X142-A11、620I-X138-G12、620I-X142-A10、620I-X138-D03、620I-X137-C08、620I-X142-E02、620I-X136-E05、620I-X138-B06、620I-X136-A09、620I-X138-A06、620I-X137-A10、620I-X139-B10、620I-X136-A04、620I-X138-D06、620I-X136-C11、620I-X138-B07、620I-X136-A02、620I-X139-G02、

620I-X136-B07、620I-X138-E03、620I-X136-G05、620I-X139-D12、620I-X136-A01、620I-X138-C12、620I-X136-G10、620I-X138-D05、620I-X136-F07、620I-X138-A01、620I-X142-E09、620I-X138-D11、620I-X136-C05、620I-X142-A02、620I-X136-C04、620I-X138-F02、620I-X136-G04、620I-X139-G12、620I-X136-B11、620I-X142-D04、620I-X136-D06、620I-X139-A01、620I-X136-D12、620I-X138-F05、620I-X136-A11、620I-X139-E05、620I-X136-C12和620I-X138-E05。

[0142] 双特异性抗体的制备

[0143] 本领域已知的任何合适的方法,例如标准重组技术,可用于制备本文所述的双特异性抗体。实施例提供如下。

[0144] 合适的亲本抗体的重链和轻链基因可以通过常规技术(例如从合适的来源PCR扩增)获得。在一个实施例中,编码对靶抗原具有特异性的单克隆抗体的DNA可很容易地被分离,并利用常规程序(例如通过利用能够特异性地结合至编码单克隆抗体的重链和轻链的基因的寡核苷酸探针)测序。细胞,诸如杂交瘤细胞,可作为这类DNA的来源。在另一个实施例中,编码对靶抗原具有特异性的单克隆抗体的DNA的序列可以例如从数据库或其他公众可用的来源获得,并且DNA可以是合成的。也可以通过用感兴趣的抗原筛选的合适的抗体文库而获得亲本抗体基因。

[0145] 因此获得的抗体重链和轻链基因可按照常规技术被分析以鉴定互补决定区(CDR)。本文所述的双特异性抗体中的任何多肽可通过常规重组技术制备,并被插入到合适的表达载体中用于在合适的宿主细胞中生产。

[0146] 编码本文所述的双特异性抗体的一条或多条多肽的核苷酸序列可被克隆到表达载体中,每条核苷酸序列可操作地与合适的启动子连接。可替代地,核苷酸序列可以可操作地与单启动子连接,以便两条序列从相同的启动子表达。在一些实施例中,两条多肽的表达通过共同的启动子控制。在其他实施例中,两条多肽的每一条的表达在不同启动子的控制下。在另一个可选实施例中,编码两条多肽的核苷酸序列被克隆到两个载体中,所述载体可以被引入到相同细胞中或不同细胞中。当这两条多肽在不同的细胞中表达时,它们中的每一条可从表达它们的宿主细胞分离,并且这两条分离的重链可在适合条件下混合和孵育,以允许双特异性抗体的形成。

[0147] 通常,编码双特异性抗体的一条链或所有链的核酸序列可被克隆到利用本领域已知的方法与合适的启动子可操作地连接的合适的表达载体中。例如,核苷酸序列和载体可以在合适的条件下与限制性内切酶接触,以在每个分子上产生互补性端部,所述互补性端部可以彼此配对并且用连接酶连接在一起。可替代地,合成的核酸连接体可以被连接到基因的末端。这些合成的连接体包含对应于载体中特定的限制酶切位点的核酸序列。表达载体/启动子的选择取决于用于产生抗体的宿主细胞的类型。

[0148] 多种启动子可用于本文所述的双特异性抗体的表达,包括但不限于巨细胞病毒(CMV)中间体早期启动子(intermediate early promoter)、诸如鲁斯氏肉瘤病毒LTR、HIV-LTR、HTLV-LTR的病毒性LTR、猿猴病毒40(SV40)早期启动子、大肠杆菌(E.coli)lac UV5启动子和单纯性疱疹tk病毒启动子。

[0149] 也可以使用可调节的启动子。这类可调节的启动子包括利用来自大肠杆菌的lac阻遏物作为转录调节剂以调节携带lac操纵子的哺乳动物细胞启动子的转录的那些可调节

的启动子[Brown,M.等,Cell,49:603-612(1987)]、利用四环素阻遏物(tetR)的那些可调节的启动子[Gossen,M.,和Bujard,H.,Proc.Natl.Acad.Sci.USA 89:5547-5551(1992);Yao,F.等,Human Gene Therapy,9:1939-1950(1998);Shockett,P.,等,Proc.Natl.Acad.Sci.USA,92:6522-6526(1995)]。其他系统包括FK506二聚体、VP16或p65(其利用雌二醇(astradiol)、RU486、二酚米乐甾酮(diphenolmurislerone)或雷帕霉素(rapamycin)。可诱导的系统可从Invitrogen、Clontech和Ariad获得。

[0150] 可以使用可调节的启动子,包括阻遏物与操纵子。在一个实施方案中,来自于大肠杆菌的lac阻遏物可作为转录调节剂调节携带lac操纵子的哺乳动物细胞启动子的转录[M.Brown等,Cell,49:603-612(1987)];Gossen和Bujard(1992);[M.Gossen等,Natl.Acad.Sci.USA,89:5547-5551(1992)]将四环素阻遏物(tetR)与转录激活剂(VP16)组合以产生tetR-哺乳动物细胞转录激活剂融合蛋白tTa(tetR-VP16),其具有来源于人巨细胞病毒(hCMV)主要即刻早期早期启动子(major immediate-early promoter)的携带tetO的最小启动子以产生tetR-tet操纵子系统来控制哺乳动物细胞中的基因表达。在一个实施方案中,使用四环素可诱导开关。四环素阻遏物(tetR)单独地,而不是tetR-哺乳动物细胞转录因子融合衍生物,可作为有效的反式调制剂,以在四环素操纵子正确地布置于CMV启动子的TATA元件的下游时调节哺乳动物细胞中的基因表达(Yao等,Human Gene Therapy)。该四环素可诱导开关的一个具体的优势是不需要使用四环素阻遏物-哺乳动物细胞反式激活剂或阻遏物融合蛋白(其在一些情况下对细胞可能是有毒的)(Gossen等,Natl.Acad.Sci.USA,89:5547-5551(1992);Shockett等,Proc.Natl.Acad.Sci.USA,92:6522-6526(1995))来实现其调节效果。

[0151] 另外,载体可包含例如以下的一些或所有:可选择的标记基因,诸如用于在哺乳动物细胞中选择稳定转染子或瞬时转染子的新霉素基因;来自人CMV的即刻早期基因的增强子/启动子序列,用于高水平转录;来自SV40的转录终止信号和RNA加工信号,用于mRNA稳定性;SV40多瘤复制起点和ColE1,用于适当的游离基因复制;内部核糖体结合位点(IRESes),通用型多克隆位点;和T7和SP6启动子,用于有义和反义RNA的体外转录。用于生产包含转基因的合适的载体和方法在本领域是已知的,并且是可以得到的。

[0152] 对实践本文所述的方法有用的多腺苷酸化信号的实例包括但不限于人胶原I多腺苷酸化信号、人胶原II多腺苷酸化信号和SV40多腺苷酸化信号。

[0153] 本公开的其他方面涉及用于制备双特异性抗体的方法,包括:在允许第一多肽和第二多肽表达的条件下培养本文所述的宿主细胞或宿主细胞组;和分离包含第一多肽和第二多肽的双特异性抗体。在一些实施方案中,宿主细胞包含表达载体,所述表达载体包含编码本文所述的第一多肽的第一核苷酸序列和编码本文所述的第二多肽的第二核苷酸序列。

[0154] 用于制备本文所述的双特异性抗体的合适的宿主细胞可以是本领域已知可用于蛋白质生产的任何宿主细胞,包括但不限于细菌细胞、酵母细胞、昆虫细胞、植物细胞或哺乳动物细胞。本文所述的双特异性抗体可以在细菌细胞(例如大肠杆菌细胞)中生产。可替代地,双特异性抗体可在真核细胞中生产。在一个实施方案中,抗体在酵母细胞(诸如毕赤酵母属(Pichia)(见例如,Powers等,2001,J.Immunol.Methods.251:123-35)、汉逊氏酵母属(Hansenula)或酵母属(Saccharomyces))中表达。在另外一个实施方案中,双特异性抗体可在哺乳动物细胞中生产。用于表达抗体的哺乳动物宿主细胞包括但不限于293细胞(见例

如ATCC CRL-1573,American Type Culture Collection® (美国典型培养物保藏中心)和Expi293F™细胞,Life Technologies™(美国生命技术公司)、中国仓鼠卵巢(CHO细胞)(包括dhfr-CHO细胞,在Urlaub和Chasin,1980,Proc.Natl.Acad.Sci.USA 77:4216-4220中描述,与DHFR选择性标记一起使用,例如在Kaufman和Sharp,1982,Mol.Biol.159:601-621中所述)、淋巴细胞系(例如NS0骨髓瘤细胞和SP2细胞、COS细胞)和来自转基因动物(例如转基因哺乳动物)的细胞。例如,细胞是乳腺上皮细胞。

[0155] 在用于本文所述的双特异性抗体的重组表达的示例性系统中,编码双特异性抗体中的两条多肽的重组表达载体通过磷酸钙介导的转染被引入到dhfr⁻CHO细胞中。在重组表达载体中,编码两条多肽的核酸可操作地与增强子/启动子调控元件(例如来源于SV40、CMV、腺病毒等,诸如CMV增强子/AdMLP启动子调控元件或SV40增强子/AdMLP启动子调控元件)连接以驱动高水平的基因转录。重组表达载体也携带DHFR基因,其允许已经利用甲氨蝶呤选择/扩增被载体转染的CHO细胞的选择。培养选择的转化子宿主细胞以允许两条多肽的表达。因此形成的四聚体分子可以从培养基中回收。另外一个用于重组表达的示例性系统在实施例2中描述。

[0156] 使用标准的分子生物学技术制备重组表达载体,转染宿主细胞,选择转化子,培养宿主细胞和从培养基中回收抗体。例如,一些抗体可以通过用蛋白质A或蛋白质G耦合基质进行亲和层析而被分离。

[0157] 双特异性抗体的用途

[0158] 本文所述的双特异性抗体或编码核酸或核酸组可用于诊断和治疗的目的。它们也可以用作基础研究和治疗研究中的研究工具。

[0159] (i) 药物组合物

[0160] 本文所述的双特异性抗体(或编码核酸或核酸组)可以与药学上可接受的载体(赋形剂),包括缓冲剂,混合,以形成用于治疗目标疾病的药物组合物。“可接受的”意指载体必需与组合物的活性成分相容(并且优选地,能够稳定活性成分),并且对待治疗的受试者是无害的。药学上可接受的赋形剂(载体),包括缓冲剂,是本领域所熟知的。见例如Remington:The Science and Practice of Pharmacy第20版(2000)Lippincott Williams和Wilkins,Ed.K.E.Hoover。

[0161] 待在本发明的方法中使用的药物组合物可包含以冻干制剂形式或水溶液形式的药学上可接受的载体、赋形剂或稳定剂(Remington:The Science and Practice of Pharmacy第20版(2000)Lippincott Williams和Wilkins,Ed.K.E.Hoover)。可接受的载体、赋形剂或稳定剂在使用的剂量和浓度下对接受者是无毒的,其可包括缓冲剂,诸如磷酸盐、柠檬酸盐和其他有机酸;抗氧化剂,包括抗坏血酸和蛋氨酸;防腐剂(诸如十八烷基二甲基苄基氯化铵、氯化六羟季铵、苯扎氯铵、苄索氯铵;苯酚、丁基或苄醇;对羟基苯甲酸烷基酯类诸如对羟基苯甲酸甲酯或对羟基苯甲酸丙酯;邻苯二酚;间苯二酚;环己醇;3-戊醇;和间甲酚);低分子量(约少于10个残基)多肽;蛋白质,诸如血清白蛋白、明胶或免疫球蛋白;亲水性聚合物,诸如聚乙烯吡咯烷酮;氨基酸诸如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸;单糖类、二糖类和其他糖类,包括葡萄糖、甘露糖或葡聚糖的;螯合剂诸如EDTA;糖类,诸如蔗糖、甘露醇、海藻糖或山梨醇;形成盐的抗衡离子诸如钠;金属复合物(例如Zn-蛋白质复合物);和/或非离子表面活性剂诸如TWEEN™、PLURONICS™或聚乙二醇

(PEG)。

[0162] 在一些实施例中,本文所述的药物组合物包含脂质体,所述脂质体包含双特异性抗体,可以通过本领域已知的方法制备,诸如在Epstein,等,Proc.Natl.Acad.Sci.USA 82:3688(1985);Hwang,等,Proc.Natl.Acad.Sci.USA 77:4030(1980);和美国专利号4,485,045和4,544,545中所述。在美国专利号5,013,556中公开了脂质体具有延长的循环时间。特别有用的脂质体可由反相蒸发法用包含磷脂酰胆碱、胆固醇和PEG衍生化的磷脂酰乙醇胺(PEG-PE)的脂质组合物产生。脂质体通过具有定义的孔隙尺寸的过滤器挤出,以产生具有预期直径的脂质体。

[0163] 双特异性抗体或编码核酸也可以被包在例如通过凝聚技术或界面聚合法在胶体药物输送系统(例如脂质体、白蛋白微球、微乳剂、纳米粒子和纳米胶囊)或在粗乳状剂中制备的微胶囊中,例如分别是羟甲基纤维素或明胶微胶囊和聚-(甲基丙烯酸甲酯)微胶囊。这类技术在本领域是已知的,见例如Remington,The Science and Practice of Pharmacy第20版,Mack Publishing(2000)。在其他实施例中,本文所述的药物组合物可以以缓释形式被配制。缓释制剂的合适的实例包括含有抗体的固体疏水性聚合物的半渗透性基质,该基质的形式是具有一定形状的制品,例如膜或微胶囊。缓释基质的实例包括聚酯、水凝胶(例如聚(2-羟乙基-甲基丙烯酸酯)或聚乙烯醇、聚乳酸(美国专利号3,773,919)、L-谷氨酸和7-乙基-L-谷氨酸酯的共聚物、不可降解的乙烯-醋酸乙烯酯、可降解的乳酸-乙醇酸共聚物,诸如LUPRON DEPOT™(由乳酸-乙醇酸共聚物和醋酸亮丙瑞林组成的可注射的微球)、蔗糖醋酸异丁酸酯和聚-D-(-)-3-羟丁酸。

[0164] 用于体内施用的药物组合物必须是无菌的。这容易通过例如经无菌过滤膜过滤而实现。治疗用抗体组合物通常被放置在具有无菌入口的容器中,例如静脉内溶液袋或具有塞子的小瓶,该塞子可被皮下注射针头穿透。

[0165] 本文所述的药物组合物可以是单位剂型,诸如片剂、丸剂、胶囊剂、粉剂、颗粒剂、溶液或悬液、或栓剂,用于口服、肠胃外或直肠施用,或用于通过吸入或吹入施用。为了制备固体组合物诸如片剂,主要活性成分可以与药物载体(例如常规的压片成分,诸如玉米淀粉、乳糖、蔗糖、山梨醇、滑石、硬脂酸、硬脂酸镁、磷酸氢钙或树胶)和其他药物稀释剂例如水混合以形成含有本发明的化合物或其无毒的药学上可接受的盐的均匀混合物的固体预制剂组合物。当提到这些预制剂组合物是均匀的时,意指活性成分均匀地分散在整个组合物中,以便组合物可以容易地被再分成相等的有效单位剂型诸如片剂、丸剂和胶囊剂。然后该固体预制剂组合物被再分成含有从0.1到约500mg的本发明的活性成分的上述类型的单位剂型。新型组合物的片剂或丸剂可被包衣或以其他方式被化合,以提供带来长效优点的剂型。例如,片剂或丸剂可包含内剂量组分和外剂量组分,后者的形式为前者上的包膜。这两种组分可以被肠溶层隔开,所述肠溶层用于抵抗胃中的崩解,从而允许内组分完整地进入十二指肠或延迟释放。多种材料可用于这类肠溶层或包衣,这类材料包括多种聚合酸和聚合酸与这类材料如虫胶、鲸蜡醇和醋酸纤维素的混合物。

[0166] 合适的表面活性剂包括(尤其包括)非离子剂诸如聚氧乙烯脱水山梨糖醇(例如Tween™ 20、40、60、80或85)和其他脱水山梨糖醇类(例如Span™ 20、40、60、80或85)。具有表面活性剂的组合物适宜地包含0.05%至5%之间的表面活性剂,其可以是0.1%至2.5%之间。应该理解,如果必要的话,可以添加其他成分例如甘露醇或其他药学上可接受的载

体。

[0167] 合适的乳剂可以利用商业上可获得的脂肪乳剂(诸如英脱利匹特TM(IntralipidTM)、乐补欣TM(LiposynTM)、InfonutrolTM、力保肪宁TM(LipofundinTM)和LipiphysanTM)制备。活性成分可以在预先混合的乳剂组合物中溶解,或可替代地,活性成分可在油(例如大豆油、红花油、棉花籽油、芝麻油、玉米油或杏仁油)和与磷脂(例如卵磷脂、大豆磷脂或大豆卵磷脂)混合后形成的乳剂和水中的溶解。应该理解,可以添加其他成分例如甘油或葡萄糖,以调节乳剂的张力。合适的乳剂通常包含多达20%的油,例如在5%和20%之间。脂肪乳剂可包含0.1至1.0.μm之间的脂肪滴,尤其是0.1至0.5.μm之间的脂肪滴,并且具有5.5至8.0的pH范围。

[0168] 乳剂组合物可以是通过使双特异性抗体和英脱利匹特TM或其组分(大豆油、卵磷脂、甘油和水)混合而制备的那些乳剂组合物。

[0169] 用于吸入或吹入的药物组合物包括药学上可接受的水或有机溶剂或其混合物中的溶液或悬液。液体或固体组合物可包含上述合适的药学上可接受的赋形剂。在一些实施方案中,为了局部或全身作用,组合物通过口腔或鼻呼吸途径施用。

[0170] 优选的无菌的药学上可接受的溶剂中的组合物可用气体雾化。雾化的溶液可以直接从雾化器中呼吸,或雾化器可以附着在面罩、帐篷(tent)或间歇式正压呼吸机上。从以适当的方式递送制剂的设备,可优选地经口腔或鼻施用溶液、悬液或粉剂组合物。

[0171] (ii) 疾病治疗

[0172] 本文所述的双特异性抗体(或编码核酸或核酸组)可用于治疗与双特异性抗体结合的一个或两个抗原相关的疾病或病症。例如,如果双特异性抗体能够结合至pKa1和FXIIa并阻断pKa1和FXIIa的活性,则其可以用于治疗与接触激活系统的调节异常相关的疾病,例如HAE和血栓形成。

[0173] HAE(包括I型、II型和III型HAE)是特征在于在例如四肢、面部、肠道和气道处严重肿胀反复发作的病症。HAE发作可由轻微创伤或应激触发。由于HAE疾病发作而导致的肠道肿胀可引起严重的腹痛、恶心和呕吐而使。气道内的肿胀可限制呼吸而导致呼吸道威胁生命的阻塞。

[0174] 血栓形成(例如静脉血栓形成或动脉血栓形成)是指血管内血凝块的形成,其可阻塞循环系统中血液的流动。血栓形成可包括与心房颤动、DVT、肺栓塞、中风或其他动脉或静脉血栓形成事件相关的心血管形成。

[0175] 为了实践本文所公开的方法,可将有效量的上述药物组合物通过合适的途径(诸如静脉内施用,例如作为丸剂或通过在规定时间内连续输注,经由肌肉内、腹膜内、脑脊髓内、皮下、关节内、滑膜内、鞘内、口腔、吸入或局部途径)施用至需要治疗的受试者(例如人类)。用于液体制剂的商业上可用的雾化器(包括喷气式雾化器和超声雾化器)可用于给药。液体制剂可直接被雾化,并且冻干粉末在重构后可以被雾化。可替代地,本文所述的双特异性抗体可利用碳氟化合物制剂和定量雾化吸入器被气雾化或作为冻干粉末和磨碎的粉末被吸入。

[0176] 经本文所述的方法治疗的受试者可以是哺乳动物,更优选地是人。哺乳动物包括但不限于农场动物、运动动物、宠物、灵长类动物、马、狗、猫、小鼠和大鼠。需要治疗的人受试者可以是患有目标疾病/病症(诸如HAE或血栓形成)的、处于患目标疾病/病症风险的或

被怀疑患有目标疾病/病症的人患者。在一些实施方案中,血栓形成与心房颤动、深部静脉血栓形成(DVT)、肺栓塞、中风或动脉或静脉血栓形成事件相关。患有目标疾病或病症的受试者可以通过常规医学检验(例如实验室测试、器官功能测试、CT扫描或超声)而被鉴定。被怀疑患有任何这类疾病/病症的受试者可能表现出该疾病/病症的一种或多种症状。处于该疾病/病症风险的受试者可以是具有该疾病/病症的一种或多种风险因素的受试者。

[0177] 本文所用的“有效量”是指单独或与一种或多种其它活性剂组合时为受试者提供治疗效果所需的每种活性剂的量。如本领域技术人员所认识到的,有效量的变化取决于所治疗的具体病状、病状的严重程度、个体患者参数(包括年龄、身体状况、尺寸、性别和体重)、治疗的持续时间、同步治疗的性质(如果有的话)、具体给药途径以及在健康从业者知识和专业内的类似的因子。这些因子是本领域普通技术人员所熟知的,并且可仅仅通过常规实验而得到处理。通常优选使用单组分或其组合的最大剂量,即根据合理医学判断的最高安全剂量。然而,本领域普通技术人员将会理解,由于医疗原因、心理原因或几乎任何其它原因,患者可能坚持使用较低剂量或可耐受剂量。

[0178] 经验上的考虑,诸如半衰期通常会有助于剂量的确定。例如,可以使用与人免疫系统相容的抗体(诸如人源化抗体或完全人抗体)来延长抗体的半衰期并防止抗体被宿主的免疫系统攻击。可以在治疗过程中确定和调整给药频率,其通常(但不一定)基于目标疾病/病症的治疗和/或抑制和/或改善和/或延迟。可替代地,双特异性抗体的持续连续释放制剂可能是合适的。用于实现缓释的各种制剂和装置是本领域已知的。

[0179] 在一个实施例中,本文所述的双特异性抗体的剂量可以在被给予一次或多次抗体施用的个体中经经验确定。给予个体增量剂量的拮抗剂。为了评估拮抗剂的功效,可以随后施用疾病/病症的指示剂。

[0180] 通常,为了施用本文所述的任何抗体,初始候选剂量可以为约2mg/kg。为了本公开的目的,典型的日剂量的范围可以为约0.1 μ g/kg至3 μ g/kg至30 μ g/kg至300 μ g/kg至3mg/kg、至30mg/kg至100mg/kg或更多中的任何一个,这取决于上述因素。对于几天内或更长时间内的重复施用(取决于病状),治疗持续到发生所期望的症状抑制或持续到达到足够的治疗水平以减轻目标疾病或病症或其症状为止。示例性的投药方案包括施用约2mg/kg的初始剂量,随后每周维持剂量为约1mg/kg的抗体,或随后每隔一周维持剂量为约1mg/kg。然而,其他剂量方案可能是有用的,这取决于从业者希望实现的药代动力学衰减的模式。例如,考虑每周投药一次至四次。在一些实施方案中,可以使用的投药范围为约3 μ g/mg至约2mg/kg(诸如约3 μ g/mg、约10 μ g/mg、约30 μ g/mg、约100 μ g/mg、约300 μ g/mg、约1mg/kg和约2mg/kg)。在一些实施方案中,投药频率是每周一次、每2周一次、每4周一次、每5周一次、每6周一次、每7周一次、每8周一次、每9周一次或每10周一次;或每月一次、每2个月一次或每3个月一次或更长时间一次。通过常规技术和测定容易地监测该疗法的进展。投药方案(包括使用的抗体)可随时间变化。

[0181] 在一些实施方案中,对于正常体重的成年患者,可施用约0.3至5.00mg/kg范围的剂量。具体的剂量方案,即剂量、时间和重复,将取决于具体个体和该个体的病史以及各种药剂的性质(诸如药剂的半衰期以及本领域熟知的其它考虑)。

[0182] 为了本公开的目的,本文所述的双特异性抗体的合适剂量将取决于所使用的特异性抗体(或其组合物)、疾病/病症的类型和严重程度、抗体是用于预防目的被施用还是用于

治疗目的被施用、以前的治疗、患者的临床病史和对拮抗剂的反应以及主治医师的判断。通常,临床医生将施用双特异性抗体,直到达到实现期望结果的剂量。一种或多种双特异性抗体的施用可以是连续的或间歇的,这取决于例如接受者的生理状况、施用的目的是治疗性还是预防性的以及熟练从业者已知的其它因素。双特异性抗体的施用可以在预选的时间段内基本上连续,或可以是一系列间隔剂量,例如在发展目标疾病或病症之前、期间或之后。

[0183] 如本文所用,术语“治疗”是指将包含一种或多种活性剂的组合物应用或施用于具有目标疾病或病症、具有该疾病/病症的症状或对该疾病/病症易感的受试者,目的是治疗、治愈、减轻、缓解、改变、医治、改善、改良或影响病症、疾病的症状或对疾病或病症的易感。

[0184] 减轻目标疾病/病症包括延缓疾病的发展或进展,或降低疾病严重程度。减轻疾病并不一定需要有疗效的结果。如其中所使用的,“延迟”目标疾病或病症的发展意味着推迟、阻碍、减缓、妨碍、稳定和/或延缓疾病进展。这种延迟可以有不同的时间长度,这取决于疾病的历史和/或被治疗的个体。一种“延迟”或减轻疾病发展或延迟疾病发病的方法是,与未使用该方法比较时,在给定的时间范围内降低发展一种或多种疾病症状的可能性和/或在给定的时间范围内降低症状程度的方法。这种比较通常基于临床研究,其使用足以给出统计学显著结果的多个受试者。

[0185] 疾病的“发展”或“进展”意指疾病的初始表现和/或随后的进展。可以使用本领域熟知的标准临床技术检测和评估疾病的发展。然而,发展也指可能无法检测的进展。为了本公开的目的,发展或进展是指症状的生物学过程。“发展”包括发生、复发和发病(onset)。如本文所用,目标疾病或病症的“发病”或“发生”包括初始发病和/或复发。

[0186] 在一些实施方案中,将本文所述的双特异性抗体以足以在体内抑制一种或两种靶抗原的活性至少20%(例如30%、40%、50%、60%、70%、80%、90%或更高)的量施用于需要治疗的受试者。在其它实施方案中,抗体以有效降低一种或两种靶抗原的水平至少20%(例如,30%、40%、50%、60%、70%、80%、90%或更高)的量被施用。

[0187] 根据待治疗的疾病的类型或疾病的部位,可以使用医学领域的普通技术人员已知的常规方法向受试者施用药物组合物。该组合物还可以通过其它常规途径施用,例如经口服、肠胃外、通过吸入喷雾、局部、直肠、鼻、口腔、阴道或经由植入的储库给药。本文所用的术语“肠胃外”包括皮下、皮内、静脉内、肌肉内、关节内、动脉内、滑膜内、胸骨内、鞘内、病灶内和颅内注射或输注技术。此外,其可以通过可注射的贮库(depot)给药途径施用于受试者,诸如使用1-、3-或6月贮库可注射的或可生物降解的材料和方法。

[0188] 可注射组合物可含有各种载体诸如植物油、二甲基乳酰胺、二甲基甲酰胺、乳酸乙酯、碳酸乙酯、肉豆蔻酸异丙酯、乙醇和多元醇(甘油、丙二醇、液体聚乙二醇等)。对于静脉内注射,可以通过滴注法施用水溶性抗体,由此输注含有抗体和生理学上可接受的赋形剂的药物制剂。生理学上可接受的赋形剂可包括例如5%右旋糖、0.9%生理盐水、林格氏溶液或其它合适的赋形剂。肌肉内制剂例如抗体的合适的可溶性盐形式的无菌制剂可以在药物赋形剂例如注射用水、0.9%生理盐水或5%葡萄糖溶液中被溶解并施用。

[0189] 在一个实施方案中,通过位点特异性或靶向局部递送技术施用双特异性抗体。位点特异性或靶向局部递送技术的实例包括双特异性抗体的各种可植入的贮库源或局部递送导管(诸如输注导管、留置导管或针导管)、合成移植物,外膜包裹物,分流器和支架或其它可植入的设备、位点特异性载体、直接注射或直接应用。见例如PCT公开号WO 00/53211和

美国专利号5,981,568。

[0190] 还可以使用含有反义多核苷酸、表达载体或次基因组多核苷酸的治疗组合物的靶向递送。受体介导的DNA递送技术在以下中描述:例如Findeis等,Trends Biotechnol. (1993) 11:202;Chiou等,Gene Therapeutics:Methods And Applications Of Direct Gene Transfer (J.A.Wolff,编辑) (1994);Wu等,J.Biol.Chem. (1988) 263:621;Wu等,J.Biol.Chem. (1994) 269:542;Zenke等,Proc.Natl.Acad.Sci.USA (1990) 87:3655;Wu等,J.Biol.Chem. (1991) 266:338。

[0191] 含有多核苷酸(例如编码本文所述的双特异性抗体的那些)的治疗组合物在约100ng至约200mg的DNA范围内被施用,用于在基因治疗方案中局部施用。在一些实施方案中,约500ng至约50mg、约1 μ g至约2mg、约5 μ g至约500 μ g和约20 μ g至约100 μ g的DNA浓度范围或更大的浓度范围也可在基因治疗方案中使用。

[0192] 本文所述的治疗性多核苷酸和多肽可以使用基因递送载体递送。基因递送载体可以是病毒或非病毒来源的(一般见Jolly,Cancer Gene Therapy (1994) 1:51;Kimura,Human Gene Therapy (1994) 5:845;Connelly,Human Gene Therapy (1995) 1:185;和Kaplitt,Nature Genetics (1994) 6:148)。可以使用内源性哺乳动物或异源性启动子和/或增强子来诱导这类编码序列的表达。编码序列的表达可以是组成型的或调控型的。

[0193] 用于递送期望的多核苷酸和在期望的细胞中表达的基于病毒的载体是本领域熟知的。基于病毒的载体的实例包括但不限于重组逆转录病毒(见例如PCT公开号WO 90/07936;WO 94/03622;WO 93/25698;WO 93/25234;WO 93/11230;WO 93/10218;WO 91/02805;美国专利号5,219,740和4,777,127;英国专利号2,200,651;和欧洲专利号0 345 242)、基于甲病毒属的载体(例如辛德毕斯病毒(Sindbis virus)载体,塞姆利基森林病毒(Semliki forest virus) (ATCC VR-67;ATCC VR-1247)、罗斯河病毒(Ross River virus) (ATCC VR-373;ATCC VR-1246)和委内瑞拉马脑炎病毒(ATCC VR-923;ATCC VR-1250;ATCC VR 1249;ATCC VR-532)和腺伴随病毒(AAV)载体(见例如PCT公开号WO 94/12649;WO 93/03769;WO 93/19191;WO 94/28938;WO 95/11984和WO 95/00655)。也可应用Curiel, Hum.Gene Ther. (1992) 3:147中所述的连接至杀伤的腺病毒的DNA的施用。

[0194] 也可以使用非病毒递送载体和方法,包括但不限于与杀伤的腺病毒单独连接或未连接的聚阳离子缩合DNA(见例如Curiel, Hum.Gene Ther. (1992) 3:147);配体连接的DNA(见例如Wu,J.Biol.Chem. (1989) 264:16985);真核细胞递送载体细胞(见例如美国专利号5,814,482;PCT公开号WO 95/07994;WO 96/17072;WO 95/30763和WO 97/42338)和核电荷中和或与细胞膜融合。也可以使用裸DNA。示例性的裸DNA引入方法在PCT公开号WO 90/11092和美国专利号5,580,859中描述。可以作为基因递送载体的脂质体在美国专利号5,422,120;PCT公开号WO 95/13796;WO 94/23697;WO 91/14445和欧洲专利号0524968中描述。另外的方法在Philip,Mol.Cell.Biol. (1994) 14:2411和Woffendin, Proc.Natl.Acad.Sci. (1994) 91:1581中描述。

[0195] 在本文描述的方法中使用的特定剂量方案,即剂量、时间和重复,将取决于特定受试者和该受试者的病史。在一些实施方案中,可将多于一种双特异性抗体或双特异性抗体与另一种合适的治疗剂的组合施用于需要治疗的受试者。双特异性抗体也可以与用于增强和/或补充药剂的有效性的其它药剂连同使用。

[0196] 目标疾病/病症的治疗效果可以通过本领域熟知的方法进行评估。

[0197] 用于治疗目标疾病的试剂盒

[0198] 本公开还提供了用于减轻目标疾病或病症的试剂盒。这类试剂盒可以包括包含本文所述的一种或多种双特异性抗体和/或一种或多种分离的核酸或核酸组的一个或多个容器。

[0199] 在一些实施方案中,试剂盒可以包括根据本文所述的任何方法使用的说明书。所包括的说明书可以包含对双特异性抗体的施用的描述,以治疗、延缓发病或减轻目标疾病诸如HAE或血栓形成。该试剂盒可以进一步包括关于基于鉴定该个体是否患有目标疾病来选择适合于治疗的个体的描述。在其它实施方案中,说明书包括关于向处于目标疾病风险的个体施用抗体的描述。

[0200] 涉及使用本文所述的双特异性抗体的说明书通常包括关于预期治疗的剂量、投药方案和给药途径的信息。容器可以是单位剂量、批量包装(例如,多剂量包装)或亚单位剂量。在本发明的试剂盒中提供的说明书通常是在标签或包装插入物(例如,试剂盒中包括的纸张)上的书面说明书,但是机器可读说明书(例如,磁性或光学存储磁盘上携带的说明书)也是可以接受的。

[0201] 标签或包装插入物指示组合物用于治疗、延缓发病和/或减轻目标疾病或病症。可以提供说明书来实施本文所述的任何方法。

[0202] 本发明的试剂盒是在合适的包装内。合适的包装包括但不限于小瓶、瓶子、罐、软包装(例如密封的聚酯薄膜(Mylar)或塑料袋)等。还考虑用于与特定装置组合使用的包装,诸如吸入器、鼻给药装置(例如雾化器)或诸如微型泵的输注装置。试剂盒可以具有无菌入口(例如,容器可以是静脉内溶液袋或具有可被皮下注射针穿透的塞子的小瓶)。容器还可以具有无菌入口(例如,容器可以是静脉内溶液袋或具有可被皮下注射针穿透的塞子的小瓶)。组合物中至少一种活性剂是如本文所述的双特异性抗体。

[0203] 试剂盒可以任选地提供额外的组分,诸如缓冲物和解释信息。通常,该试剂盒包括容器和在容器上或与容器连接的标签或包装插入物。在一些实施方案中,本发明提供了包括上述试剂盒的内容物的制品。

[0204] 常规技术

[0205] 除非另有说明,本发明的实践将采用在本领域技术范围内的分子生物学(包括重组技术)、微生物学、细胞生物学、生物化学和免疫学的常规技术。这些技术在文献中得到充分解释,所述文献诸如Molecular Cloning:A Laboratory Manual,第二版(Sambrook,等,1989)Cold Spring Harbor Press;Oligonucleotide Synthesis(M.J.Gait,编辑,1984);Methods in Molecular Biology,Humana Press;Cell Biology:A Laboratory Notebook(J.E.Cellis,编辑,1998)Academic Press;Animal Cell Culture(R.I.Freshney,编辑,1987);Introduction to Cell and Tissue Culture(J.P.Mather和P.E.Roberts,1998)Plenum Press;Cell and Tissue Culture:Laboratory Procedures(A.Doyle,J.B.Griffiths和D.G.Newell,编辑,1993-8)J.Wiley and Sons;Methods in Enzymology(Academic Press,Inc.);Handbook of Experimental Immunology(D.M.Weir和C.C.Blackwell,编辑);Gene Transfer Vectors for Mammalian Cells(J.M.Miller和M.P.Calos,编辑,1987);Current Protocols in Molecular Biology(F.M.Ausubel,等,编

辑,1987);PCR:The Polymerase Chain Reaction, (Mullis,等,编辑,1994);Current Protocols in Immunology(J.E.Coligan等,编辑,1991);Short Protocols in Molecular Biology (Wiley和Sons,1999);Immunobiology (C.A.Janeway和P.Travers,1997);Antibodies (P.Finch,1997);Antibodies:a practical approach (D.Catty.,编辑,IRL Press,1988-1989);Monoclonal antibodies:a practical approach (P.Shepherd和C.Dean,编辑,Oxford University Press,2000);Using antibodies:a laboratory manual (E.Harlow和D.Lane (Cold Spring Harbor Laboratory Press,1999);The Antibodies (M.Zanetti和J.D.Capra,编辑,Harwood Academic Publishers,1995)。

[0206] 在没有进一步的详细阐述的情况下,相信本领域技术人员可以基于上述描述最大限度地利用本发明。因此,以下具体实施方案将被解释为仅仅是说明性的,而不是以任何方式限制本公开的其余部分。本文引用的所有出版物通过引用并入本文,用于本文的目的或用于本文所涉及的主题。

[0207] 实施例1:结合pKal和因子XIIa的示例性双特异性抗体的构建和表征

[0208] 使用DX-2930和抗FXIIa克隆559C-M0071-F06、559C-M0184-B04、559C-M0179-D04、559C-M0181-C02和559C-M0180-G03中的一个作为亲本抗体,构建了一些示例性抗pKal/抗FXIIa双特异性抗体,包括克隆X0120-A01、X0120-C01、X0120-E01、X0120-G01、X0121-A03、X0121-C01、X0121-E01、X0121-G01、X0122-A01和X0122-C01。见下表2:

[0209] 表2:示例性双特异性抗体的组分

双特异性抗体克隆	抗 pKal 部分	抗 FXIIa 部分
X0120-A01	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0184-B04 (H→L)的 scFv
X0120-C01	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0184-B04 (L→H)的 scFv
X0120-E01	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0179-D04 (H→L)的 scFv
X0120-G01,	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0179-D04 (L→H)的 scFv
X0121-A03	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0181-C02 (H→L)的 scFv
X0121-C01	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0181-C02 (L→H)的 scFv
X0121-E01	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0180-G03 (H→L)的 scFv
X0121-G01	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0180-G03 (L→H)的 scFv
X0122-A01,	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0071-F06 (H→L)的 scFv
X0122-C01	DX-2930 (IgG)	融合至 DX-2930 的重链的 C-末端的克隆 559C-M0071-F06 (L→H)的 scFv

[0211] 在抗FXIIa克隆中,559C-M0071-F06是亲本克隆,559C-M0184-B04从HCDR1+2亲和力和成熟中获得,且559C-M0179-D04、559C-M0181-C02和559C-M0180-G03是从轻链亲和力成熟中获得的克隆。

[0212] 上述表2中列出的所有示例性双特异性抗体克隆是四价分子,其包含四条多肽链,包括DX-2930的轻链的两条多肽链(上文提供的SEQ ID NO:10),和与FXIIa克隆之一的scFv链融合的DX-2930的重链的两条融合多肽链(不包括恒定链的铰链结构域中的赖氨酸残基)。5种抗FXIIa克隆中的每一个的scFv链均以重-轻(H→L)方向和轻-重(L→H)方向合成。在scFv链的所有实施例,使用内部(GGGGS)₄连接体(SEQ ID NO:23)。构建scFv,以便轻-重方向的克隆含有初始的两个氨基酸(RT),所述氨基酸在连接体序列开始之前开始(initiate)恒定区。重-轻方向的克隆在终止密码子之前仅含有来自轻链恒定区的第一个氨基酸(R)。

[0213] 示例性双特异性抗体的每一个的融合多肽的氨基酸序列提供如下:

[0214] 双特异性抗体克隆X0120-A01重链-ScFv融合物(SEQ ID NO:11):

[0215] MGWSCIIILFLVATATGAHSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEA
LHNHYTQKSLSLSPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSFYSMHWVRQAPGKGLEWVSRIYPSGG
VTKYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGGS
GGGGSGGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLHLSNGYNYLDWYLQKPGQSPQLLIYLSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGGQGTKVEIKR

[0216] 双特异性抗体克隆X0120-C01重链-ScFv融合物(SEQ ID NO:12):

[0217] MGWSCIIILFLVATATGAHSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEA
LHNHYTQKSLSLSPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLHLSNGYNYLDWYLQKPGQSPQLLIYLG
SNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGGQGTKVEIKRTGGGGSGGGSGGGSGGGG
SEVQLLESGGGLVQPGGSLRLSCAASGFTFSFYSMHWVRQAPGKGLEWVSRIYPSGGVTKYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSS

[0218] 双特异性抗体克隆X0120-E01重链-ScFv融合物(SEQ ID NO:13):

[0219] MGWSCIIILFLVATATGAHSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFC SVMHEA
LHNHYTQKSLSLSPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSGYIMAWVRQAPGKGLEWVSRIYPSGG

ITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSSGGGGSGGGGS
GGGGSGGGGSDIQMTQSPLSLSVAPGEPASISCRSSQSLLRNGHNYLDWYLQKPGQSPQLLIYLG SNRASGVPERF
SGSGSGTDFTLRISRVEAEDVGVYYCMQALQARTFGQGTKVEIKR

[0220] 双特异性抗体克隆X0120-G01重链-ScFv融合物(SEQ ID NO:14):

[0221] MGWSCIIILFLVATATGAHSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEA
LHNHYTQKSLSLSPGKSGGGSDIQMTQSPLSLSVAPGEPASISCRSSQSLLRNGHNYLDWYLQKPGQSPQLLIYLG
SNRASGVPERFSGSGSGTDFTLRISRVEAEDVGVYYCMQALQARTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGS
EVQLLESGGGLVQPGGSLRLSCAASGFTFSGYIMAWVRQAPGKGLEWVSYIYPSGGITVYADSVKGRFTISRDN SKN
TLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSS

[0222] 双特异性抗体克隆X0121-A03重链-ScFv融合物(SEQ ID NO:15):

[0223] MGWSCIIILFLVATATGAHSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEA
LHNHYTQKSLSLSPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSGYIMAWVRQAPGKGLEWVSYIYPSGG
ITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSSGGGGSGGGGS
GGGGSGGGGSDIQMTQSPLSLPVTGPGEASISCRSSQSLHSGNYLDWYLQKPGQSPQLLIYLG SNRASGV PDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTRTFGQGTKVEIKR

[0224] 双特异性抗体克隆X0121-C01重链-ScFv融合物(SEQ ID NO:16):

[0225] MGWSCIIILFLVATATGAHSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEA
LHNHYTQKSLSLSPGKSGGGSDIQMTQSPLSLPVTGPGEASISCRSSQSLHSGNYLDWYLQKPGQSPQLLIYLG
SNRASGV PDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTRTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGS
EVQLLESGGGLVQPGGSLRLSCAASGFTFSGYIMAWVRQAPGKGLEWVSYIYPSGGITVYADSVKGRFTISRDN SKN
TLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSS

[0226] 双特异性抗体克隆X0121-E01重链-ScFv融合物(SEQ ID NO:17):

[0227] MGWSCIIILFLVATATGAHSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG

PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA
LHNHYTQKSLSLSPGKSGGGSEVQLLES GGGGLVQP GGGSLRLSCAASGFTFSGYIMAWVRQAPGKGLEWVS YIYPSGG
ITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGGS
GGGGSGGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLHLSNGYNYLDWYLQKPGQSPQIMIIYLG SNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPRTFGQGTKVEIKR

[0228] 双特异性抗体克隆X0121-G01重链-ScFv融合物(SEQ ID NO:18):

[0229] MGWSCIIILFLVATATGAHSEVQLLES GGGGLVQP GGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA
LHNHYTQKSLSLSPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLHLSNGYNYLDWYLQKPGQSPQIMIIYLG
SNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPRTFGQGTKVEIKRTGGGGSGGGSGGGSGGGG
SEVQLLES GGGGLVQP GGGSLRLSCAASGFTFSGYIMAWVRQAPGKGLEWVS YIYPSGGITVYADSVKGRFTISRDN SK
NTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSS

[0230] 双特异性抗体克隆X0122-A01重链-ScFv融合物(SEQ ID NO:19):

[0231] MGWSCIIILFLVATATGAHSEVQLLES GGGGLVQP GGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA
LHNHYTQKSLSLSPGKSGGGSEVQLLES GGGGLVQP GGGSLRLSCAASGFTFSGYIMAWVRQAPGKGLEWVS YIYPSGG
ITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGGS
GGGGSGGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLHLSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKR

[0232] 双特异性抗体克隆X0122-C01重链-ScFv融合物(SEQ ID NO:20):

[0233] MGWSCIIILFLVATATGAHSEVQLLES GGGGLVQP GGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSG
IYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYI
CNVNHKPSNTKVDKRVEPKSCDTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEA
LHNHYTQKSLSLSPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLHLSNGYNYLDWYLQKPGQSPQLLIYLG
SNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGG

SEVQLLESGGGLVQPGGSLRLSCAASGFTFSGYIMAWVRQAPGKGLEWVSYIYPSGGITVYADSVKGRFTISRDNK
NTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTSS

[0234] 为了构建上述示例性双特异性抗体的表达盒,将DX-2930的重链和轻链的编码序列克隆到pRh1-CHO载体中,用与scFv编码序列连接的C-末端SGGS连接体修饰。连接区含有用于有效克隆scFv的BamHI限制性酶切位点。选择5个抗因子XIIa克隆,通过BamHI/XbaI限制性酶切位点插入构建体中。

[0235] 上文提供的序列的斜体部分是指信号肽。本文公开的双特异性抗体的抗pKa1部分可以包含相同的信号肽,或者可以被去除信号肽或用不同的信号肽替换信号肽。用于产生分泌蛋白的信号肽是本领域熟知的。

[0236] 编码双特异性抗体的核苷酸序列(以顺-反操纵子形式)提供如下:

[0237] X0120-A01 (SEQ ID NO:24)

[0238] ATGGGATGGTCCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGCCTCCGACATCCAGATG
ACCCAGTCCCCCTCCACCCTGTCCGCTCTGTGGGCGACAGAGTGACCATCACCTGTGGGCTCCAGTCCATCTC
CAGCTGGCTGGCTGGTATCAGCAGAAGCCCGCAAGGCCCCCAAGCTGCTGATCTACAAGGCCAGCACCTGGAAT
CCGGCGTGCCCTCCAGATTCTCCGGCTCTGGCTCCGGCACCGAGTTCACCCTGACCATCAGCTCCCTGCAGCCCGAC
GACTTCGCCACCTACTACTGCCAGCAGTACAACACCTACTGGACCTTCGGCCAGGGCACCAAGGTGGAAATCAAGCG
GACCGTGGCCGCTCCCTCCGTGTTTCATCTTCCACCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTCT
GCCTGCTGAACAACTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCC
CAGGAATCCGTGACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCTTACCCTGACCCTGTCCAAGGCCGA
CTACGAGAAGCACAAGGTGTACGCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGTCTTCAACC
GGGGCGAGTGCTGATGAGGCGCGCTTCGCGTCGAGCATGCATCTAGGGCGGCCAATTCGCCCCCTCTCCCCCCCC
CCCCTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTG
CCGTCTTTTGGCAATGTGAGGGCCCCGAAACCTGGCCCTGTCTTCTGACGAGCATTCCTAGGGGTCTTTCCCTCT
CGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGT
CTGTAGCGACCCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAA
GATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAGAGTCAAATGGCTCTC
TTCAAGCGTATTCAACAAGGGGTGAAGGATGCCCAGAAGGTACCCATTGTATGGGATCTGATCTGGGGCTCGGT
GCAGATGCTTTACATGTGTTTAGTCGAGGTAAAAAAGCTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTCT
TGAAAAACACGATGATAATATGGCCACAACCATGGGATGGTCTGCATCATCCTGTTTCTGGTGGCCACAGCCACAG
GCGCTCACTCCGAGGTGCAATTGCTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGC
GCCGCTCCGGCTTACCTTCTCCCACTACATCATGATGTGGGTGCGACAGGCTCCTGGCAAGGGGCTGGAATGGGT
GTCCGGCATCTACTCCTCCGGCGGCATACCGTGTACGCCGACTCCGTGAAGGGCCGGTTACCATCTCTCGGGACA
ACTCCAAGAACACCCTGTACCTGCAGATGAACTCCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCTACCGG
CGGATCGGCGTGCCAGACGGGACGAGTTCGACATCTGGGGGAGGGACCATGGTGACAGTGTCTCCGCTCCAC
CAAGGGCCCCCTCTGTGTTCCCGCTAGCACCTCCAGCAAGTCCACCTCCGGCGGCACCGCTGCTCTGGGCTGCCTCG
TCAAGGACTACTTCCCCGAGCCCGTGACCGTGTCTGGAACCTCTGGCGCCCTGACCAGCGGAGTGCATACCTTCCCT
GCCGTGCTCCAGTCTCCGGCCTGTACAGCCTGTCTCTGTCTGACCGTGCCCTCCAGCTCCCTGGGCACCCAGAC
CTACATCTGCAACGTGAACCAAGCCCTCCAACACCAAAGTGGACAAGCGGGTGAACCCAAGTCTGCGACACCC
ACACCTGTCCCCCTTGCCCTGCCCTGAACTGCTGGGCGGACCCAGCGTGTTCCTGTTCCCCCAAGCCCAAGGAC

ACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCACGAGGACCCTGAAGTGAAGTT
TAATTGGTACGTGGACGGCGTGGAAGTGCATAACGCCAAGACCAAGCCCAGAGAGGAACAGTACAACCTCCACCTACC
GGGTGGTGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAGTACAAGTGCAAGGTGTCCAACAAG
GCCCTGCCTGCCCCCATCGAAAAGACCATCAGCAAGGCCAAGGGCCAGCCTCGCGAGCCCCAGGTGTACACCCTGCC
CCCTAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCCTCCGATATCG
CCGTGGAATGGGAGTCCAACGGCCAGCCCCGAGAACAACCTACAAGACCACCCCCCTGTGCTGGACAGCGACGGCTCA
TTCTTCTGTACTCCAAGCTGACCGTGGAACAAGTCCCGGTGGCAGCAGGGCAACGTGTTCTCCTGCTCCGTGATGCA
CGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGTCCCTGTCTCCCGCAAGTCTGGCGGAGGATCCGAAGTGC
AGCTGCTGGAAGCGGGCAGGCTGGTGACGCTGGAGGCAGCCTGAGACTGTCTTGCCTGCCAGCGGCTTACC
TTCAGCTTCTACAGCATGCACTGGGTCCGACAGGCTCCAGGCAAGGGCCTGGAATGGGTGTCCCGGATCTACCCCTC
TGGCGGCGTGACCAAATACGCCGACAGCGTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCCTGT
ACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCACCCGGCAGCGGTACAGAGGCCCAAG
TACTACTACTACATGGACGTGTGGGGCAAGGGCACAACCGTGACCGTGTCTAGCGGAGGCGGAGGATCTGGCGGAGG
TGGAAGTGGTGGTGGCGGAAGTGGCGGAGGCGGCAGCGACATCCAGATGACCCAGAGCCCCCTGAGCCTGCCCCTGA
CACCTGGCGAGCCTGCCAGCATCAGCTGCAGAAGCAGCCAGAGCCTGCTGCACAGCAACGGCTACAACCTACCTGGAC
TGGTATCTGCAGAAGCCCGGCCAGTCCCCCAGCTGCTGATCTACCTGGGCAGCAACAGAGCCAGCGGCGTGCCCGA
CAGATTACGCGGCAGCGGCTCCGGCACCGACTTCAACCCTGAAGATCAGCCGGGTGGAAGCCGAGGACGTGGGCGTGT
ACTATTGCATGCAGGCCCTGCAGACCCCTGGACCTTCGGCCAGGGCACCAAGGTGGAATCAAGAGATGAATCTAG
A

[0239] X0120-C01 (SEQ ID NO:25)

[0240] ATGGGATGGTCCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGCACTCCGACATCCAGATG
ACCCAGTCCCCCTCCACCCTGTCCGCCTCTGTGGGCGACAGAGTGACCATCACCTGTGGGCTCCAGTCCATCTC
CAGCTGGCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGCCCCCAAGCTGCTGATCTACAAGGCCAGCACCTGGAAT
CCGGCGTGCCCTCCAGATTCTCCGGCTCTGGCTCCGGCACCGAGTTCACCCTGACCATCAGCTCCCTGCAGCCCGAC
GACTTCGCCACCTACTACTGCCAGCAGTACAACACCTACTGGACCTTCGGCCAGGGCACCAAGGTGGAATCAAGCG
GACCGTGGCCGCTCCCTCCGTGTTTCATCTTCCCACCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTCT
GCCTGCTGAACAACCTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCC
CAGGAATCCGTGACCGAGCAGGACTCCAAGGACAGCACCTACTCCCTGTCTCTACCCTGACCCTGTCCAAGGCCGA
CTACGAGAAGCACAAGGTGTACGCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGTCTTCAACC
GGGCGAGTGCTGATGAGGCGCGCTTCGCGTCGAGCATGCATCTAGGGCGGCCAATTCGCCCCCTCTCCCCCCCCC
CCCCTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTG
CCGTCTTTTGGCAATGTGAGGGCCCCGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCTCT
CGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGT
CTGTAGCGACCCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAA
GATACACCTGCAAAGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAGAGTCAAATGGCTCTC
TTCAAGCGTATTCAACAAGGGCTGAAGGATGCCAGAAGTACCCATTGTATGGGATCTGATCTGGGGCTCGGT
GCAGATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTT
TGAAAAACACGATGATAATATGGCCACAACCATGGGATGGTCCTGCATCATCCTGTTTCTGGTGGCCACAGCCACAG
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CAAGGGCCCCCTCTGTGTTCCCGCTAGCACCTCCAGCAAGTCCACCTCCGGCGGCACCGCTGCTCTGGGCTGCCTCG
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CTACATCTGCAACGTGAACCACAAGCCCTCCAACACCAAAGTGGACAAGCGGGTGAACCCAAGTCTGCGACACCC
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ACCCTGATGATCTCCCGGACCCCCGAAGTGACCTGCGTGGTGGTGGACGTGTCCACGAGGACCCTGAAGTGAAGTT
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GGGTGGTGTCCGTGCTGACCGTGTGACCCAGGACTGGCTGAACGGCAAAGAGTACAAGTGAAGGTGTCCAACAAG
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CCCTAGCCGGGAAGAGATGACCAAGAACCAGGTGTCCCTGACCTGTCTGGTCAAGGGCTTCTACCCCTCCGATATCG
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CAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGCACCCGGCAGC
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TAGA

[0241] X0120-E01 (SEQ ID NO:26)

[0242] ATGGGATGGTCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGCACTCCGACATCCAGATG
ACCCAGTCCCCCTCCACCCTGTCCGCTCTGTGGGCGACAGAGTGACCATCACCTGTGGGCCTCCAGTCCATCTC
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GACTTCGCCACCTACTACTGCCAGCAGTACAACACCTACTGGACCTTCGGCCAGGGCACCAAGGTGGAATCAAGCG
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TGGTATCTGCAGAAGCCCGGCCAGTCCCCCAGCTGCTGATCTACCTGGGCGAGCAACAGAGCCAGCGGCGTGCCGA
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[0243] X0120-G01 (SEQ ID NO:27)

[0244] ATGGGATGGTCCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGCACTCCGACATCCAGATG
ACCCAGTCCCCCTCCACCCTGTCCGCTCTGTGGGCGACAGAGTGACCATCACCTGTGGGCTCCAGTCCATCTC

CAGCTGGCTGGCCTGGTATCAGCAGAAGCCCCGCAAGGCCCCCAAGCTGCTGATCTACAAGGCCAGCACCTGGAAT
CCGGCGTGCCCTCCAGATTCTCCGGCTCTGGCTCCGGCACCAGTTACCCCTGACCATCAGCTCCCTGCAGCCCCGAC
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GACCGTGGCCGCTCCCTCCGTGTTTCATCTTCCCACCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTCT
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CTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGTCTTTCAACC
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CGGCTCTGAGGTGCAGCTGCTGGAATCTGGCGGCGGACTGGTGCAGCCTGGCGGCAGCCTGAGACTGTCTTGCGCCG
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[0245] X0121-A03 (SEQ ID NO:28)

[0246] ATGGGATGGTCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGACTCCGACATCCAGATG
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[0247] X0121-C01 (SEQ ID NO:29)

[0248] ATGGGATGGTCCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGCCTCCGACATCCAGATG
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[0249] X0121-E01 (SEQ ID NO:30)

[0250] ATGGGATGGTCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGCACTCCGACATCCAGATG
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GACCGTGGCCGCTCCCTCCGTGTTTCATCTTCCACCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTCT
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CTACGAGAAGCACAAGGTGTACGCCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGTCCTTCAACC
GGGCGAGTGCTGATGAGGCGCGCCTTCGCGTCGAGCATGCATCTAGGGCGGCCAATTCGGCCCCCTCTCCCCCCCC
CCCCTAACGTTACTGGCCGAAGCCGCTTGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTG
CCGTCTTTTGGCAATGTGAGGGCCCCGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCTCT
CGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGT
CTGTAGCGACCCCTTTGCAGGCAGCGGAACCCCCACCTGGCGACAGGTGCCTCTGCGGCCAAAAGCCACGTGTATAA
GATACACCTGCAAAGGCGGCACAACCCACGTGCCACGTTGTGAGTTGGATAGTTGTGGAAGAGTCAAATGGCTCTC

TTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCAGAAGGTACCCCATTTGTATGGGATCTGATCTGGGGCCTCGGT
GCAGATGCTTTACATGTGTTTAGTCGAGGTTAAAAAACGTCTAGGCCCCCGAACCACGGGGACGTGGTTTTCTCT
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GCGCTCACTCCGAGGTGCAATTGCTGGAATCCGGCGGAGGACTGGTGCAGCCTGGCGGCTCCCTGAGACTGTCTTGC
GCCGCTCCGGCTTACCTTCTCCCACTACATCATGATGTGGGTGCGACAGGCTCCTGGCAAGGGGCTGGAATGGGT
GTCCGGCATCTACTCCTCCGGCGGCATCACCGTGTACGCCGACTCCGTGAAGGGCCGGTTCACCATCTCTCGGGACA
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ACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCACCCGGCAGCGGTACAGAGGCCCAAG
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[0251] X0121-G01 (SEQ ID NO:31)

[0252] ATGGGATGGTCCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGCCTCCGACATCCAGATG
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CAGCTGGCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGGCCCCAAGCTGCTGATCTACAAGGCCAGCACCTGGAAT
CCGGCGTGCCCTCCAGATTCTCCGGCTCTGGCTCCGGCACCGAGTTCACCCTGACCATCAGCTCCCTGCAGCCCGAC
GACTTCGCCACCTACTACTGCCAGCAGTACAACACCTACTGGACCTTCGGCCAGGGCACCAAGGTGGAATCAAGCG
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GCCTGCTGAACAACCTTCTACCCCCGCGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCCCTGCAGTCCGGCAACTCC
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CTACGAGAAGCACAAGGTGTACGCTGCGAAGTGACCCACCAGGGCCTGTCCAGCCCCGTGACCAAGTCTTCAACC
GGGCGAGTGCTGATGAGGCGCGCTTCGCGTCGAGCATGCATCTAGGGCGGCCAATTCGCCCCCTCTCCCCCCCC
CCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTG
CCGTCTTTTGGCAATGTGAGGGCCCCGAAACCTGGCCCTGTCTTCTTGACGAGCATTCCTAGGGGTCTTTCCCTCT
CGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGT
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GATACACCTGCAAAGGGCGGCACAACCCAGTGCCACGTTGTGAGTTGGATAGTTGTGGAAAGAGTCAAATGGCTCTC
TTCAAGCGTATTCAACAAGGGGCTGAAGGATGCCCAGAAGGTACCCATTGTATGGGATCTGATCTGGGGCCTCGGT
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CAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGCACCCGGCAGC

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TAGA

[0253] X0122-A01 (SEQ ID NO:32)

[0254] ATGGGATGGTCCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGCACTCCGACATCCAGATG
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GACTTCGCCACCTACTACTGCCAGCAGTACAACACCTACTGGACCTTCGGCCAGGGCACCAAGGTGGAAATCAAGCG
GACCGTGGCCGCTCCCTCCGTGTTTCATCTTCCCACCTCCGACGAGCAGCTGAAGTCCGGCACCGCCTCCGTGGTCT
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CGCCAAAGGAATGCAAGGTCTGTTGAATGTCGTGAAGGAAGCAGTTCCTCTGGAAGCTTCTTGAAGACAAACAACGT
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GCCGCTCCGGCTTACCTTCTCCCACTACATCATGATGTGGGTGCGACAGGCTCCTGGCAAGGGGCTGGAATGGGT
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[0255] X0122-C01 (SEQ ID NO:33)

[0256] ATGGGATGGTCTGCATCATCCTGTTTCTGGTGGCTACAGCCACAGGCGTGCCTCCGACATCCAGATG
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CCCCTAACGTTACTGGCCGAAGCCGCTTGGAATAAGGCCGGTGTGCGTTTGTCTATATGTTATTTTCCACCATATTG
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 GGCACCAAGGTGGAAATCAAGCGGACAGGCGGCGGAGGCTCTGGCGGAGGTGGAAGCGGAGGCGGAGGAAGTGGCGG
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 CCGCCAGCGGCTTCACCTTCAGCGGCTACATCATGGCCTGGGTCCGACAGGCCCTGGCAAGGGCCTGGAATGGGTG
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 CAGCAAGAACACCCTGTACCTGCAGATGAACAGCCTGCGGGCCGAGGACACCGCCGTGTACTATTGCACCCGGCAGC
 GGTACAGAGGCCCAAGTACTACTACTACATGGACGTGTGGGGCAAGGGCACCACCGTGACCGTGTCCAGCTGAATC
 TAGA

[0257] 产生编码上述双特异性抗体的pRh1表达质粒。经0.2um无菌过滤后,将质粒转染到Expi293F™细胞的60mL d1培养物中,使用ExpiFectamine™作为转染试剂在Expi293™表达培养基中培养,如LifeTech方案(Life Technologies™,加利福尼亚卡尔斯巴德)所述。在LifeTech方案中描述的培养的第2天加入Expifectamine™转染增强剂1和2。培养物在37℃、8%CO₂、140rpm孵育7天。通过离心收获培养物,然后进行0.2um无菌过滤,并在4℃储存。使用蛋白A柱分批纯化克隆。

[0258] 将不同浓度的双特异性抗体与单独的FXIIa和pKa1样品一起孵育,并且通过测量共价连接到肽底物的化学部分的荧光的变化来随着时间监测这些蛋白酶裂解肽底物的能力。该动力学数据的斜率等于酶促蛋白水解速率,然后针对抑制剂的浓度将其作图。然后通过非线性回归将所得到的图拟合到紧密结合抑制剂方程(tight binding inhibitor equation)(方程式1)以获得表观抑制常数(K_i^{app})。

$$[0259] \quad v = V_0 \cdot \frac{([E] - [I] - K_i^{app}) + \sqrt{([E] - [I] - K_i^{app})^2 - 4[E] \cdot K_i^{app}}}{2} \quad [\text{方程式 1}]$$

[0260] 图1和图2显示了测试的每种双特异性抗体的pKa1和FXIIa抑制活性的图。所有测试的克隆都能够抑制pKa1和FXIIa二者。每种双特异性抗体的 K_i^{app} pKa1和FXIIa列在下面表3中。

[0261] 表3. 双特异性抗体的表观抑制常数

[0262]	双特异性抗体	K_i^{app} pKa1 (nM)	K_i^{app} FXIIa (nM)

X120-A01	0.1376+/-0.0206	0.0515+/-0.0186
X121-E01	0.1593+/-0.0245	0.6114+/-0.0714
X122-A01	0.1693+/-0.0242	6.0467+/-0.6497
X122-C01	0.1610+/-0.0221	5.6900+/-0.6512
对照M71-F06IgG	N/A	0.8758+/-0.0579

[0263] 实施例2:结合pKa1和因子XIIa的示例性双特异性抗体的构建和表征

[0264] 如下构建了另外一组示例性抗pKa1/抗FXIIa双特异性抗体。分子的IG部分与实施例1中所用的相同,即DX-2930。对于抗FXIIa组分,选择36个分离株,并将其转变为轻链/重链和重链/轻链方向的scFv。使用SGGS (SEQ ID NO:22) 连接体将scFv融合到DX-2930IgG。当构建scFv时,使用(G₄S)₄连接体将抗FXIIa可变重链和可变轻链结构域彼此融合。以下提供双特异性抗体的序列。

[0265] 构建的双特异性分子显示出与先前对于DX-2930测定的值大体一致的抗pKa1活性(表4)。一些值显示针对pKa1较弱的效力,可能是由于计算浓度的错误,或可能由于聚集。scFv组分的抗FXIIa活性通常低于先前测定的值,这可能是由于与scFv相关的固有不稳定性(表4)。血浆测定中双特异性分子的活性显示了超过DX-2930和抗FXIIa IgG的显著改善。该测定中DX-2930显示的范围在70-100nM之间,而抗FXIIa亲本抗体显示在 $\sim$ 100nM范围的抑制。测试的双特异性分子的图显示在1-10nM范围内的抑制(表5)。

[0266] 表4. 针对各自的靶标的72种双特异性抗pKa1+抗FXIIa抗体的表观K_i。DX-2930和FXIIa主要候选物(559C-M0292-D07)被用作对照。

[0267]

	ScFv 方向	双特异性分离株	FXII-名称	抗-pKal 校正的 Ki, app (pM)	抗-FXIIa 校正的 Ki, app (pM)
1	H→L	620I-X136-C0 7	559C-M0177-B1 1	182	699
	L→H	620I-X138-A0 8	559C-M0177-B1 1	920	357
2	H→L	620I-X136-B0 2	559C-M0177-C1 2	210	2857
	L→H	620I-X139-A1 2	559C-M0177-C1 2	2053	4084
3	H→L	620I-X137-B0 8	559C-M0178-A0 8	312	409
	L→H	620I-X142-A0 4	559C-M0178-A0 8	162	1233

[0268]

4	H→L	620I-X142-B1 1	559C-M0179-A0 3	622	7719
	L→H	620I-X138-B0 1	559C-M0179-A0 3	169	6628
5	H→L	620I-X136-C0 1	559C-M0182-B0 4	173	957
	L→H	620I-X138-A1 2	559C-M0182-B0 4	405	1925
6	H→L	620I-X136-A1 2	559C-M0182-D0 4	234	304
	L→H	620I-X138-A0 2	559C-M0182-D0 4	206	288
7	H→L	620I-X136-A0 5	559C-M0182-H0 1	179	111
	L→H	620I-X138-C0 7	559C-M0182-H0 1	196	314
8	H→L	620I-X136-E0 7	559C-M0182-H0 4	190	312
	L→H	620I-X142-B0 2	559C-M0182-H0 4	156	955
9	H→L	620I-X136-F1 1	559C-M0183-B1 2	201	235
	L→H	620I-X142-A0	559C-M0183-B1	160	2140

[0269]

		5	2		
1 0	H→L	620I-X136-C0 9	559C-M0183-C0 3	173	90
	L→H	620I-X138-B1 0	559C-M0183-C0 3	75	58
11	H→L	620I-X136-C0 8	559C-M0183-D0 8	216	1231
	L→H	620I-X139-A1 1	559C-M0183-D0 8	235	3835
1 2	H→L	620I-X136-D0 5	559C-M0183-H0 8	55	13
	L→H	620I-X138-D0 4	559C-M0183-H0 8	215	79
1 3	H→L	620I-X136-G0 8	559C-M0184-B0 4	176	28
	L→H	620I-X142-B0 7	559C-M0184-B0 4	224	775
1 4	H→L	620I-X142-A1 1	559C-M0184-D0 1	158	195
	L→H	620I-X138-G1 2	559C-M0184-D0 1	186	766
1 5	H→L	620I-X142-A1 0	559C-M0184-E0 6	175	389

[0270]

	L→H	620I-X138-D0 3	559C-M0184-E0 6	79	344
1 6	H→L	620I-X137-C0 8	559C-M0184-F1 2	153	34
	L→H	620I-X142-E0 2	559C-M0184-F1 2	162	186
1 7	H→L	620I-X136-E0 5	559C-M0191-A0 3	158	172
	L→H	620I-X138-B0 6	559C-M0191-A0 3	330	405
1 8	H→L	620I-X136-A0 9	559C-M0191-B1 1	190	X
	L→H	620I-X138-A0 6	559C-M0191-B1 1	145	X
1 9	H→L	620I-X137-A1 0	559C-M0191-C0 9	195	205
	L→H	620I-X139-B1 0	559C-M0191-C0 9	247	189
2 0	H→L	620I-X136-A0 4	559C-M0191-E0 4	171	230
	L→H	620I-X138-D0 6	559C-M0191-E0 4	199	132
2	H→L	620I-X136-C1	559C-M0191-E0	154	38

[0271]

1		1	9		
	L→H	620I-X138-B0 7	559C-M0191-E0 9	246	135
2 2	H→L	620I-X136-A0 2	559C-M0191-H0 9	176	136
	L→H	620I-X139-G0 2	559C-M0191-H0 9	171	161
2 3	H→L	620I-X136-B0 7	559C-M0191-H1 0	168	99
	L→H	620I-X138-E0 3	559C-M0191-H1 0	178	122
2 4	H→L	620I-X136-G0 5	559C-M0192-A0 1	179	100
	L→H	620I-X139-D1 2	559C-M0192-A0 1	428	383
2 5	H→L	620I-X136-A0 1	559C-M0192-A0 3	135	224
	L→H	620I-X138-C1 2	559C-M0192-A0 3	267	697
2 6	H→L	620I-X136-G1 0	559C-M0192-D0 2	171	28
	L→H	620I-X138-D0 5	559C-M0192-D0 2	519	139

[0272]

2		620I-X136-F0	559C-M0192-D1		
7	H→L	7	2	183	167
		620I-X138-A0	559C-M0192-D1		
	L→H	1	2	154	465
2		620I-X142-E0	559C-M0192-F0		
8	H→L	9	1	174	163
		620I-X138-D1	559C-M0192-F0		
	L→H	1	1	178	443
2		620I-X136-C0	559C-M0192-F0		
9	H→L	5	6	150	58
		620I-X142-A0	559C-M0192-F0		
	L→H	2	6	152	63
3		620I-X136-C0	559C-M0192-F0		
0	H→L	4	7	205	189
		620I-X138-F0	559C-M0192-F0		
	L→H	2	7	464	794
3		620I-X136-G0	559C-M0192-G0		
1	H→L	4	3	179	107
		620I-X139-G1	559C-M0192-G0		
	L→H	2	3	276	252
3		620I-X136-B1	559C-M0192-G0		
2	H→L	1	5	172	184
		620I-X142-D0	559C-M0192-G0		
	L→H			170	414

[0273]

		4	5		
3		620I-X136-D0	559C-M0192-H0		
3	H→L	6	4	176	84
		620I-X139-A0	559C-M0192-H0		
	L→H	1	4	146	53
3		620I-X136-D1	559C-M0192-H1		
4	H→L	2	1	179	63
		620I-X138-F0	559C-M0192-H1		
	L→H	5	1	214	147
3		620I-X136-A1	559C-M0292-D0		
5	H→L	1	7	199	193
		620I-X139-E0	559C-M0292-D0		
	L→H	5	7	196	172
3		620I-X136-C1	559C-M0177-A0		
6	H→L	2	6	217	1567
		620I-X138-E0	559C-M0177-A0		
	L→H	5	6	186	245
3					
7	板 1		DX-2930	160	X
	板 2		DX-2930	138	X
3					
8	板 1		559C-M292-D07	X	36
	板 2		559C-M292-D07	X	38

[0274] 表5:血浆激活测定中亲本抗FXIIa分离株和抗pKa1/抗FXIIa双特异性分子的比较。血浆稀释1:40。向稀释的血浆中加入抑制剂。将2.5%鞣花酸加入血浆中。2分钟后,通过

加入玉米胰蛋白酶抑制剂猝灭激活。通过添加前荧光 (profluorescent) 底物测量 pKa1 活性。

			血浆抑制	
			IgG	双特异性
			IC50(nM)	IC50/Ki(nM)
[0275]	FXII IgG 分离株名称	双特异性分离株名称		
	559C-M0192-A03	620I-X0136-A01	514	52
	559C-M0192-F06	620I-X0136-C05	304	2.6
	559C-M0191-E09	620I-X0136-C11	31	1.8
	559C-M0192-H11	620I-X0136-D12	101	3
	559C-M0192-A01	620I-X0136-G05	198	8

[0276] 选择5种示例性候选物 (620I-X0136-D12、620I-X0136-C05、620I-X0136-C11、620I-X0136-G05和620I-X0136-A01), 作进一步分析。在这5种主要候选物 (中, 620I-X0136-A01由于低表达值和尺寸排阻色谱 (SEC) 踪迹中的多种类而被淘汰。在剩余的4个主要候选物 (中, 每个分离株含有不同程度的高分子量聚集体 (16-35%) (图3)。该聚集体被确定为浓度依赖性的, 并且假定其是通过scFv结构域相互作用的二聚体结构。

[0277] 通过血浆抑制测定评估示例性双特异性抗体620I-X0136-D12 (D12) 抑制血浆pKa1活性的能力。简言之, 在存在或不存在HMWK的情况下, 在测定缓冲液 (20mM Tris-HCl pH7.5、150mM NaCl、1mM EDTA、0.1% PEG-8000和0.1% Triton X-100) 中, 将含有大量的前pKa1和FXII的重构血浆以1:40稀释。前-pKa1、FXII和HMWK的浓度等于其在血浆中的正常浓度。在室温, 在96孔微孔板中将不同浓度的抑制剂加入到重构血浆中。然后通过加入25% (2.5%最终) 稀释的鞣花酸溶液引发接触激活, 通过轻轻摇动混合微孔板, 并在室温进行2分钟, 借以加入100nM的CTI。然后将10 μ l该混合物移至重复微孔板中, 所述微孔板含有在30 $^{\circ}$ C预平衡的80 μ l测定缓冲液。然后将该稀释板在30 $^{\circ}$ C再孵育5分钟, 并如上评估PFR-AMC的蛋白水解, 但用X轴中使用的反算的 (back-calculated) 抑制剂浓度进行曲线拟合至针对紧密结合抑制剂的修饰的莫里森 (Morrison) 方程 (在最终测定读数中, 血浆被稀释1:400)。本研究的结果显示在图5 (在存在单链HMWK的情况下) 和图6 (在不存在HMWK的情况下) 中。双特异性抗体表现优于亲本IgG的总和, 特别是在存在HMWK的情况下。使用紧密结合抑制剂方程, 在HMWK存在下, D12的表观Ki值测定为8.8nM, 在不存在HMWK的情况下测定为2.6nM。

[0278] 还评估了与抗FXIIa抗体 (D06) 和抗pKa1抗体 (H03) 相比, 双特异性抗体候选物620I-X0136-D12 (D12) 在APTT测定中延迟活化部分凝血活酶时间 (APTT) 的能力 (图7)。简言之, 以三种浓度 (25、50、100) 将抑制剂分子 (或对照稀释缓冲液=25mM HEPE、pH7.5、125mM NaCl) 以1:1混合加入到纯血浆 (neat plasma) 中, 并在37 $^{\circ}$ C预平衡5分钟。将2 $\times$ 50 μ l该混合物分配到2个单独的KC4 Δ 测定样品池 (cuvette) (带金属球) 中。60秒后, 将50 μ l aPTT试剂 (激活剂, Pacific Untasis APTT-XL) 加入到旋转的样品池中, 并在加入aPTT (在t=0秒) 后180秒加入50 μ l的CaCl₂。KC4 Δ 仪器以秒为单位记录凝结时间。

[0279] 还评估了双特异性抗体候选物620I-X0136-D12 (D12) 抑制纤维蛋白形成的能力 (图8)。

[0280] 抗体序列: 本实施例中描述的所有双特异性分子均含有第一多肽, 所述第一多肽

链在重链/轻链或轻链/重链方向上包含DX-2930重链、SGGS连接体和抗FXIIa scFv。DX-2930轻链也使用相同的载体表达。对于每个分离株只列出重链+scFv序列。

[0281] >DX-2930轻链(没有信号序列)

[0282] DIQMTQSPSTLSASVGDRTITCRASQSISSWLAWYQQKPGKAPKLLIYKASTLESGVPSRFSGSGSGT
TEFTLTITSSLPDDFATYYCQQYNTYWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFY
VQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID
NO:46)

[0283] 来源于36种示例性抗FXIIa IgG的双特异性:

[0284] >620I-X0136-C07=DX2930重链+559C-M0177-B11 L4H scFv

[0285] EVQLLESGLLVQPGGSLRLSAAAGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGLLVQPGGSLRLSAAAGFTFSRYIMVWVRQAPGKGLEWVSRIYPSGGYTRYADSVKGR
FTISRDNKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLHLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:51)

[0286] >620I-X0138-A08=DX2930重链+559C-M0177-B11 H4L scFv

[0287] EVQLLESGLLVQPGGSLRLSAAAGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLHLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGL
GLVQPGGSLRLSAAAGFTFSRYIMVWVRQAPGKGLEWVSRIYPSGGYTRYADSVKGRFTISRDNKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:52)

[0288] >620I-X0136-B02=DX2930重链+559C-M0177-C12 L4H scFv

[0289] EVQLLESGLLVQPGGSLRLSAAAGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGLLVQPGGSLRLSAAAGFTFSFYHMHVVRQAPGKGLEWVSRIYPSGGMTRYADSVKGR
FTISRDNKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGSGGGSGGGGS

DIQMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:53)

[0290] >620I-X0139-A12=DX2930重链+559C-M0177-C12 H4L scFv

[0291] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSFYHMHVVRQAPGKGLEWVSRIVPSGGMTRYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTTVTVSS (SEQ ID NO:54)

[0292] >620I-X0137-B08=DX2930重链+559C-M0178-A08 L4H scFv

[0293] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSFYIMGWVRQAPGKGLEWVSRIYPSGGATQYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:55)

[0294] >620I-X0142-A04=DX2930重链+559C-M0178-A08 H4L scFv

[0295] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSFYIMGWVRQAPGKGLEWVSRIYPSGGATQYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTTVTVSS (SEQ ID NO:56)

[0296] >620I-X0142-B11=DX2930重链+559C-M0179-A03 L4H scFv

[0297] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGLLVQPGGSLRLSQAASGFTFSGYIMAWVRQAPGKGLEWVSIIYPSGGITVYADSVKGR
FTISRDNKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYDWYLQKPGQSPQLLIYLSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDIGVYYCMQGRHRPYTFGQGTREIKR (SEQ ID NO:57)

[0298] >620I-X0138-B01=DX2930重链+559C-M0179-A03 H4L scFv

[0299] EVQLLESGLLVQPGGSLRLSQAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYDWYLQKPGQSPQLLIYLSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDIGVYYCMQGRHRPYTFGQGTREIKRTGGGGSGGGSGGGSGGGSGGGSEVQLLESGG
GLVQPGGSLRLSQAASGFTFSGYIMAWVRQAPGKGLEWVSIIYPSGGITVYADSVKGRFTISRDNKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:58)

[0300] >620I-X0136-C01=DX2930重链+559C-M0182-B04 L4H scFv

[0301] EVQLLESGLLVQPGGSLRLSQAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGLLVQPGGSLRLSQAASGFTFSWYVMQWVRQAPGKGLEWVSIIYPSGGHTKYADSVKGR
FTISRDNKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYDWYLQKPGQSPQLLIYLSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKEIKR (SEQ ID NO:59)

[0302] >620I-X0138-A12=DX2930重链+559C-M0182-B04 H4L scFv

[0303] EVQLLESGLLVQPGGSLRLSQAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQSVMEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYDWYLQKPGQSPQLLIYLSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKEIKRTGGGGSGGGSGGGSGGGSGGGSEVQLLESGG
GLVQPGGSLRLSQAASGFTFSWYVMQWVRQAPGKGLEWVSIIYPSGGHTKYADSVKGRFTISRDNKNTLYLQMNS

LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTTVTVSS (SEQ ID NO:60)

[0304] >620I-X0136-A12=DX2930重链+559C-M0182-D04 L4H scFv

[0305] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLES GGG LVQP GGS LRLSCAASGFTFSMYTMNWVRQAPGKGLEWVSRIYPSGGKTLYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTD
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:61)

[0306] >620I-X0138-A02=DX2930重链+559C-M0182-D04 H4L scFv

[0307] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLES G
GLVQP GGS LRLSCAASGFTFSMYTMNWVRQAPGKGLEWVSRIYPSGGKTLYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTTVTVSS (SEQ ID NO:62)

[0308] >620I-X0136-A05=DX2930重链+559C-M0182-H01 L4H scFv

[0309] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLES GGG LVQP GGS LRLSCAASGFTFSRYVMHWVRQAPGKGLEWVSSIWPSGGMTKYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTD
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:63)

[0310] >620I-X0138-C07=DX2930重链+559C-M0182-H01 H4L scFv

[0311] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP

REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGLVQPGGSLRLSCAASGFTFSRYVMHWVRQAPGKGLEWVSSIWPSGGMTKYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSS (SEQ ID NO:64)

[0312] >620I-X0136-E07=DX2930重链+559C-M0182-H04 L4H scFv

[0313] EVQLLES GGGLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKSGGGSEVQLLES GGGLVQPGGSLRLS CAASGFTFSWYIMGWVRQAPGKGLEWVSRIYPSGGTTFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKR (SEQ ID NO:65)

[0314] >620I-X0142-B02=DX2930重链+559C-M0182-H04 H4L scFv

[0315] EVQLLES GGGLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGLVQPGGSLRLS CAASGFTFSWYIMGWVRQAPGKGLEWVSRIYPSGGTTFYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSS (SEQ ID NO:66)

[0316] >620I-X0136-F11=DX2930重链+559C-M0183-B12 L4H scFv

[0317] EVQLLES GGGLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKSGGGSEVQLLES GGGLVQPGGSLRLS CAASGFTFSWYVMYVVRQAPGKGLEWVSRIYPSGGITHYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKR (SEQ ID NO:67)

[0318] >620I-X0142-A05=DX2930重链+559C-M0183-B12 H4L scFv

[0319] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYVMYVWRQAPGKGLEWVSRIYPSGGITHYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:68)

[0320] >620I-X0136-C09=DX2930重链+559C-M0183-C03 L4H scFv

[0321] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGGLVQPGGSLRLSCAASGFTFSWYNMHVWRQAPGKGLEWVSYISPSGGKTKYTDSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGGSGGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:69)

[0322] >620I-X0138-B10=DX2930重链+559C-M0183-C03 H4L scFv

[0323] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYNMHVWRQAPGKGLEWVSYISPSGGKTKYTDSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:70)

[0324] >620I-X0136-C08=DX2930重链+559C-M0183-D08 L4H scFv

[0325] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC

LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSRYIMGWVRQAPGKGLEWVSSIYPSGGVTRYADSVKGR
FTISRDNKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTTVTVSSGGGGSGGGSGGGSGGGG
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:71)

[0326] >620I-X0139-A11=DX2930重链+559C-M0183-D08 H4L scFv

[0327] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSRYIMGWVRQAPGKGLEWVSSIYPSGGVTRYADSVKGRFTISRDNKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTTVTVSS (SEQ ID NO:72)

[0328] >620I-X0136-D05=DX2930重链+559C-M0183-H08 L4H scFvEVQLLESGGGLVQPGGS
LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTISRDNKNTLYLQMNSLRAEDTAV
YYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALT
SGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSV
FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYK
TTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKSGGGSEVQLLESGGGLVQPGG
SLRLSCAASGFTFSRYIMHWVRQAPGKGLEWVSSIYPSGGVTKYADSVKGRFTISRDNKNTLYLQMNSLRAEDTA
VYYCTRQRYRGPKYYYYMDVWGKGTTVTVSSGGGGSGGGSGGGSGGGGSDIQMTQSPLSLPVTGPGEPAISCRS
SQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGYYCMQALQTPW
TFGQGTKVEIKR (SEQ ID NO:73)

[0329] >620I-X0138-D04=DX2930重链+559C-M0183-H08 H4L scFv

[0330] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSRYIMHWVRQAPGKGLEWVSSIYPSGGVTKYADSVKGRFTISRDNKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTTVTVSS (SEQ ID NO:74)

[0331] >620I-X0136-G08=DX2930重链+559C-M0184-B04 L4H scFv

[0332] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLES GGG LVQP GGS LRLSCAASGFTFSFYSMHWVRQAPGKGLEWVSRIYPSGGVTKYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:75)

[0333] >620I-X0142-B07=DX2930重链+559C-M0184-B04 H4L scFv

[0334] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLTKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGSGGGSEVQLLES G
GLVQP GGS LRLSCAASGFTFSFYSMHWVRQAPGKGLEWVSRIYPSGGVTKYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSS (SEQ ID NO:76)

[0335] >620I-X0142-A11=DX2930重链+559C-M0184-D01 L4H scFv

[0336] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLES GGG LVQP GGS LRLSCAASGFTFSFYVMGWVRQAPGKGLEWVSRIYPSGGLTQYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:77)

[0337] >620I-X0138-G12=DX2930重链+559C-M0184-D01 H4L scFv

[0338] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL

SPGKSGGGSDIQMTQSPLSLPVTGPGEASISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDFR
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSFYVMGWVRQAPGKGLEWVSRIYPSGGLTQYADSVKGRFTISRDN SKNTLYLQMN
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSS (SEQ ID NO:78)

[0339] >620I-X0142-A10=DX2930重链+559C-M0184-E06 L4H scFv

[0340] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMN SLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSWYVMQWVRQAPGKGLEWVSSIWPSGGKTVYADSVKGR
FTISRDN SKNTLYLQMN SLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTGPGEASISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDFRSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:79)

[0341] >620I-X0138-D03=DX2930重链+559C-M0184-E06 H4L scFv

[0342] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMN SLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEASISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDFR
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYVMQWVRQAPGKGLEWVSSIWPSGGKTVYADSVKGRFTISRDN SKNTLYLQMN
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSS (SEQ ID NO:80)

[0343] >620I-X0137-C08=DX2930重链+559C-M0184-F12 L4H scFv

[0344] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMN SLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSWYVMHWVRQAPGKGLEWVSGIWPSSGGRTKYADSVKGR
FTISRDN SKNTLYLQMN SLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTGPGEASISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDFRSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:81)

[0345] >620I-X0142-E02=DX2930重链+559C-M0184-F12 H4L scFv

[0346] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF

TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSLKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYVMHWVRQAPGKGLEWVSGIWPSSGRTKYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:82)

[0347] >620I-X0136-E05=DX2930重链+559C-M0191-A03 L4H scFv

[0348] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSLKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSQYIMHWVRQAPGKGLEWVSSIYPSSGNTKYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGGSGGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:83)

[0349] >620I-X0138-B06=DX2930重链+559C-M0191-A03 H4L scFv

[0350] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSLKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSQYIMHWVRQAPGKGLEWVSSIYPSSGNTKYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:84)

[0351] >620I-X0136-A09=DX2930重链+559C-M0191-B11 H4L scFv

[0352] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSLKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFS (SEQ ID NO:85)

[0353] >620I-X0138-A06=DX2930重链+559C-M0191-B11 H4L scFv

[0354] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFS (SEQ ID NO:86)

[0355] >620I-X0137-A10=DX2930重链+559C-M0191-C09 L4H scFv

[0356] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGLVQPGGSLRLSCAASGFTFSPYIMHWVRQAPGKGLEWVSRIYPSGGATVYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTTVTVSSGGGGSGGGGSGGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
LKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKR (SEQ ID NO:87)

[0357] >620I-X0139-B10=DX2930重链+559C-M0191-C09 H4L scFv

[0358] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSPYIMHWVRQAPGKGLEWVSRIYPSGGATVYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTTVTVSS (SEQ ID NO:88)

[0359] >620I-X0136-A04=DX2930重链+559C-M0191-E04 L4H scFv

[0360] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL

SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSMYIMHWVRQAPGKGLEWVSSIYPSGGMTKYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:89)

[0361] >620I-X0138-D06=DX2930重链+559C-M0191-E04 H4L scFv

[0362] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGSGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSMYIMHWVRQAPGKGLEWVSSIYPSGGMTKYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSS (SEQ ID NO:90)

[0363] >620I-X0136-C11=DX2930重链+559C-M0191-E09 L4H scFv

[0364] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSWYSMHWVRQAPGKGLEWVSVIYPSGGKTRYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:91)

[0365] >620I-X0138-B07=DX2930重链+559C-M0191-E09 H4L scFv

[0366] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLHNSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGSGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYSMHWVRQAPGKGLEWVSVIYPSGGKTRYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSS (SEQ ID NO:92)

[0367] >620I-X0136-A02=DX2930重链+559C-M0191-H09 L4H scFv

[0368] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF

TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLES GGLVQPGGSLRLSCAASGFTFSMYVMHWVRQAPGKGLEWVSSIYPSGGLTKYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSL LHSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRFSGSGSGTDF
TLKISRVEAEDGVYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:93)

[0369] >620I-X0139-G02=DX2930重链+559C-M0191-H09 H4L scFv

[0370] EVQLLES GGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSL LHSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRF
SGSGSGTDFTLKISRVEAEDGVYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLES GGL
GLVQPGGSLRLSCAASGFTFSMYVMHWVRQAPGKGLEWVSSIYPSGGLTKYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSS (SEQ ID NO:94)

[0371] >620I-X0136-B07=DX2930重链+559C-M0191-H10 L4H scFv

[0372] EVQLLES GGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLES GGLVQPGGSLRLSCAASGFTFSWYTMHWVRQAPGKGLEWVSSIYPSGGFTRYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSL LHSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRFSGSGSGTDF
TLKISRVEAEDGVYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:95)

[0373] >620I-X0138-E03=DX2930重链+559C-M0191-H10 H4L scFv

[0374] EVQLLES GGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSL LHSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRF

SGSGSGTDFTLKISRVEADVGVYYCMQALQTPWTFGQGTKVEIKRTGGGSGGGSGGGSGGGSGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYTMHWVRQAPGKGLEWVSSIYPSSGGFTRYADSVKGRFTISRDN SKNTLYLQMN
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSS (SEQ ID NO:96)

[0375] >620I-X0136-G05=DX2930重链+559C-M0192-A01 L4H scFv

[0376] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMN SLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYVMHWVRQAPGKGLEWVSSIYPSSGGLTKYADSVKGR
FTISRDN SKNTLYLQMN SLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSSGGGSGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRFSGSGSGTDF
TLKISRVEADVGVYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:97)

[0377] >620I-X0139-D12=DX2930重链+559C-M0192-A01 H4L scFv

[0378] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMN SLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRF
SGSGSGTDFTLKISRVEADVGVYYCMQALQTPWTFGQGTKVEIKRTGGGSGGGSGGGSGGGSGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSHYVMHWVRQAPGKGLEWVSSIYPSSGGLTKYADSVKGRFTISRDN SKNTLYLQMN
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSS (SEQ ID NO:98)

[0379] >620I-X0136-A01=DX2930重链+559C-M0192-A03 L4H scFv

[0380] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMN SLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSWYVMQWVRQAPGKGLEWVSSIYPSSGGM TKYADSVKGR
FTISRDN SKNTLYLQMN SLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSSGGGSGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLG SNRASGVPDRFSGSGSGTDF
TLKISRVEADVGVYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:99)

[0381] >620I-X0138-C12=DX2930重链+559C-M0192-A03 H4L scFv

[0382] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMN SLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT

AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYVMQWVRQAPGKGLEWVSSIYPSSGGMTKYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSS (SEQ ID NO:100)

[0383] >620I-X0136-G10=DX2930重链+559C-M0192-D02 L4H scFv

[0384] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSQYVMHWVRQAPGKGLEWVSSIWPSGGFTKYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSSGGGGSGGGGSGGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKR (SEQ ID NO:101)

[0385] >620I-X0138-D05=DX2930重链+559C-M0192-D02 H4L scFv

[0386] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSQYVMHWVRQAPGKGLEWVSSIWPSGGFTKYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSS (SEQ ID NO:102)

[0387] >620I-X0136-F07=DX2930重链+559C-M0192-D12 L4H scFv

[0388] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSVSMHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSWYIMQWVRQAPGKGLEWVSSIYPSSGGRTKYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TVTVSSGGGGSGGGGSGGGGSGGGGS

DIQMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:103)

[0389] >620I-X0138-A01=DX2930重链+559C-M0192-D12 H4L scFv

[0390] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYIMQWVRQAPGKGLEWVSSIYPSSGGRTKYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:104)

[0391] >620I-X0142-E09=DX2930重链+559C-M0192-F01 L4H scFv

[0392] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSWYNMAWVRQAPGKGLEWVSRIYPSGGMTQYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:105)

[0393] >620I-X0138-D11=DX2930重链+559C-M0192-F01 H4L scFv

[0394] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGKSGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLHSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYNMAWVRQAPGKGLEWVSRIYPSGGMTQYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:106)

[0395] >620I-X0136-C05=DX2930重链+559C-M0192-F06 L4H scFv

[0396] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGLLVQPGGSLRLSCAASGFTFSWYVMHWVRQAPGKGLEWVSSIYPSGGKTSYADSVKGR
FTISRDNKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:107)

[0397] >620I-X0142-A02=DX2930重链+559C-M0192-F06 H4L scFv

[0398] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTISWNSGALTSGVHTFPAVLQSSGLYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSWYVMHWVRQAPGKGLEWVSSIYPSGGKTSYADSVKGRFTISRDNKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:108)

[0399] >620I-X0136-C04=DX2930重链+559C-M0192-F07 L4H scFv

[0400] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTISWNSGALTSGVHTFPAVLQSSGLYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGLLVQPGGSLRLSCAASGFTFSQYVMSWVRQAPGKGLEWVSRIYPSGGVTKYADSVKGR
FTISRDNKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:109)

[0401] >620I-X0138-F02=DX2930重链+559C-M0192-F07 H4L scFv

[0402] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTISWNSGALTSGVHTFPAVLQSSGLYSLSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSQYVMSWVRQAPGKGLEWVSRIYPSGGVTKYADSVKGRFTISRDNKNTLYLQMNS

LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSS (SEQ ID NO:110)

[0403] >620I-X0136-G04=DX2930重链+559C-M0192-G03 L4H scFv

[0404] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLES GGG LVQP GGS LRLSCAASGFTFSQYNMVWVRQAPGKGLEWVSRIWPSGGKTTYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:111)

[0405] >620I-X0139-G12=DX2930重链+559C-M0192-G03 H4L scFv

[0406] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGGSEVQLLES G
GLVQP GGS LRLSCAASGFTFSQYNMVWVRQAPGKGLEWVSRIWPSGGKTTYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSS (SEQ ID NO:112)

[0407] >620I-X0136-B11=DX2930重链+559C-M0192-G05 L4H scFv

[0408] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLES GGG LVQP GGS LRLSCAASGFTFSQYTMWVRQAPGKGLEWVSRIYPSGGVTQYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTVTVSSGGGGSGGGSGGGSGGGGS
DIQMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:113)

[0409] >620I-X0142-D04=DX2930重链+559C-M0192-G05 H4L scFv

[0410] EVQLLES GGG LVQP GGS LRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP

REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKSGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGGLVQPGGSLRLSCAASGFTFSQYTMVWVRQAPGKGLEWVSRIYPSGGVTQYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSS (SEQ ID NO:114)

[0411] >620I-X0136-D06=DX2930重链+559C-M0192-H04 L4H scFv

[0412] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSQYVMHWVRQAPGKGLEWVSRIYPSGGLTNYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKR (SEQ ID NO:115)

[0413] >620I-X0139-A01=DX2930重链+559C-M0192-H04 H4L scFv

[0414] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKSGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGGLVQPGGSLRLSCAASGFTFSQYVMHWVRQAPGKGLEWVSRIYPSGGLTNYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSS (SEQ ID NO:116)

[0415] >620I-X0136-D12=DX2930重链+559C-M0192-H11 L4H scFv

[0416] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSQVMHEALHNHYTQKSLSLSPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSQYVMHWVRQAPGKGLEWVSRIYPSGGHTRYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGT TTVTVSSGGGGSGGGGSGGGGSGGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKEIKR (SEQ ID NO:117)

[0417] >620I-X0138-F05=DX2930重链+559C-M0192-H11 H4L scFv

[0418] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSQYVMHWVRQAPGKGLEWVSSIWPSSGGHTRYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDVWGKTTTVTVSS (SEQ ID NO:118)

[0419] >620I-X0136-A11=DX2930重链+559C-M0292-D07 L4H scFv

[0420] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGGLVQPGGSLRLSCAASGFTFSNYVMHWVRQAPGKGLEWVSSIWPSSGGKTKYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDAWGQGTTVTVSSGGGGSGGGGSGGGGSGGGGS
DIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:119)

[0421] >620I-X0139-E05=DX2930重链+559C-M0292-D07 H4L scFv

[0422] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRF
SGSGSGTDFTLKISRVEAEDVGVYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGGSGGGGSGGGGSEVQLLESGG
GLVQPGGSLRLSCAASGFTFSNYVMHWVRQAPGKGLEWVSSIWPSSGGKTKYADSVKGRFTISRDN SKNTLYLQMNS
LRAEDTAVYYCTRQRYRGPKYYYYMDAWGQGTTVTVSS (SEQ ID NO:120)

[0423] >620I-X0136-C12=DX2930重链+559C-M0177-A06 L4H scFv

[0424] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC

LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSFYSMHWVRQAPGKGLEWVSRIYPSGGITSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSSGGGGSGGGSGGGSGGGSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKR (SEQ ID NO:121)

[0425] >620I-X0138-E05=DX2930重链+559C-M0177-A06 H4L scFv

[0426] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSLSPGKSGGGSDIQMTQSPLSLPVTGPGEPAISCRSSQSLLSHNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISRVEAEDVGYYCMQALQTPWTFGQGTKVEIKRTGGGGSGGGSGGGSGGGSGGGSEVQLLESGGLVQPGGSLRLSCAASGFTFSFYSMHWVRQAPGKGLEWVSRIYPSGGITSYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCTRQRYRGPKYYYYMDVWGKGTITVTVSS (SEQ ID NO:122)

[0427] 实施例3:包含二硫键的示例性双特异性抗体的构建和表征

[0428] 为了对抗这些聚集体,对4个克隆,620I-X0177-A01 (620I-X0173-A11)、620I-X0177-C01 (620I-X0173-C07)、620I-X0177-E01 (620I-X0173-E07) 和620I-X0177-G01 (620I-X0173-G11),将VH残基44(C₄₄)和VL残基100(C₁₀₀)之间的二硫键工程化到scFv区中。包含具有二硫化物的scFv的双特异性抗体的SEC分析显示出高分子量峰的显著降低,使范围降至1-2%。图4。这种聚集的降低适用所有测试浓度。这些双特异性克隆的Biacore显示出与pKa1和FXIIa的紧密、特异性的结合(图9)。这些分离株的血浆抑制范围为0.5至8nM范围(图10)。

[0429] 进行如本文所述的血浆抑制测定以测定双特异性抗体620I-X0177-A01的抑制活性。将血浆稀释1:40,并将抑制剂加入到稀释的血浆中。将2.5% (最终浓度)的稀释鞣花酸溶液加入到血浆中。大约2分钟后,通过加入CTI猝灭血浆激活。通过加入本文所述的前荧光底物来测量血浆中的pKa1活性。由此获得的结果显示于图11中。

[0430] 将克隆620I-X0177-A01的抑制活性与单独的或组合的亲本抗体的抑制活性进行比较。在亲本IgG和双特异性抗体之间观察到亲和力的急下降。图12。

[0431] 此外,根据本文所述的方法评估各种双特异性抗体对APTT的能力。所有测试的抗体显示APTT的剂量依赖性延迟。图13。

[0432] 还检验了抗体克隆1A01 (抗FXIIa) 和7A01 (针对pKa1和FXII两者的双特异性) 抑制纤维蛋白沉积的能力,结果显示于图14中。观察到纤维蛋白沉积的剂量依赖性抑制。

[0433] 总体而言,酶抑制测定确定了示例性双特异性抗体620I-X0177-A01的单独的抗pKa1和抗FXIIa组分的表观K_i值与亲本分子相似,表观K_i值分别为389pM和73pM。令人惊讶的是,在接触-激活的稀释血浆方面的另外的实验显示,该双特异性抗体在预防pKa1产生方面的效力是亲本抗体的1:1组合的5倍,并且是任一种单独的亲本抗体的效力的20倍。这些数据表明双特异性抗体在其关闭(shut down)接触系统激活的正反馈环的能力方面有独特

的效力。

[0434] 下文提供了具有二硫化物限制的scFv的双特异性抗体的序列：

[0435] >620I-X0173-A11 (620I-X0177-A01) = 620I-X0136-D12种系化的+基因优化的scFv+二硫化物稳定化

[0436] EVQLLES GGG L V Q P G G S L R L S C A A S G F T F S H Y I M M W V R Q A P G K G L E W V S G I Y S S G G I T V Y A D S V K G R F
T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A Y R R I G V P R R D E F D I W G Q G T M V T V S S A S T K G P S V F P L A P S S K S T S G G T
A A L G C L V K D Y F E P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K R V
E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P
R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C
L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L
S P G K S G G G S E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S Q Y V M H W V R Q A P G K C L E W V S S I W P S G G H T R Y A D S V K G R
F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R Q R Y R G P K Y Y Y Y M D V W G Q G T T V T V S S G G G G S G G G G S G G G G S
D I V M T Q S P L S L P V T P G E P A S I S C R S S Q S L L H S N G Y N Y L D W Y L Q K P G Q S P Q L L I Y L G S N R A S G V P D R F S G S G S G T D F
T L K I S R V E A E D V G V Y Y C M Q A L Q T P W T F G C G T K V E I K R (SEQ ID NO:47)

[0437] >620I-X0173-C07 (620I-X0177-C01) = 620I-X0136-C05种系化的+基因优化的scFv+二硫化物稳定化

[0438] EVQLLES GGG L V Q P G G S L R L S C A A S G F T F S H Y I M M W V R Q A P G K G L E W V S G I Y S S G G I T V Y A D S V K G R F
T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A Y R R I G V P R R D E F D I W G Q G T M V T V S S A S T K G P S V F P L A P S S K S T S G G T
A A L G C L V K D Y F E P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K R V
E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P
R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C
L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L
S P G K S G G G S E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S W Y V M H W V R Q A P G K C L E W V S S I Y P S G G K T S Y A D S V K G R
F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R Q R Y R G P K Y Y Y Y M D V W G Q G T T V T V S S G G G G S G G G G S G G G G S G G G G S
D I V M T Q S P L S L P V T P G E P A S I S C R S S Q S L L H S N G Y N Y L D W Y L Q K P G Q S P Q L L I Y L G S N R A S G V P D R F S G S G S G T D F
T L K I S R V E A E D V G V Y Y C M Q A L Q T P W T F G C G T K V E I K R (SEQ ID NO:48)

[0439] >620I-X0173-E07 (620I-X0177-E01) = 620I-X0136-C11种系化的+基因优化的scFv+二硫化物稳定化

[0440] EVQLLES GGG L V Q P G G S L R L S C A A S G F T F S H Y I M M W V R Q A P G K G L E W V S G I Y S S G G I T V Y A D S V K G R F
T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A Y R R I G V P R R D E F D I W G Q G T M V T V S S A S T K G P S V F P L A P S S K S T S G G T
A A L G C L V K D Y F E P E P V T V S W N S G A L T S G V H T F P A V L Q S S G L Y S L S S V V T V P S S S L G T Q T Y I C N V N H K P S N T K V D K R V
E P K S C D K T H T C P P C P A P E L L G G P S V F L F P P K P K D T L M I S R T P E V T C V V V D V S H E D P E V K F N W Y V D G V E V H N A K T K P
R E E Q Y N S T Y R V V S V L T V L H Q D W L N G K E Y K C K V S N K A L P A P I E K T I S K A K G Q P R E P Q V Y T L P P S R E E M T K N Q V S L T C
L V K G F Y P S D I A V E W E S N G Q P E N N Y K T T P P V L D S D G S F F L Y S K L T V D K S R W Q Q G N V F S C S V M H E A L H N H Y T Q K S L S L
S P G K S G G G S E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S W Y S M H W V R Q A P G K C L E W V S V I Y P S G G K T R Y A D S V K G R
F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R Q R Y R G P K Y Y Y Y M D V W G Q G T T V T V S S G G G G S G G G G S G G G G S G G G G S
D I V M T Q S P L S L P V T P G E P A S I S C R S S Q S L L H S N G Y N Y L D W Y L Q K P G Q S P Q L L I Y L G S N R A S G V P D R F S G S G S G T D F
T L K I S R V E A E D V G V Y Y C M Q A L Q T P W T F G C G T K V E I K R (SEQ ID NO:49)

[0441] >620I-X0173-G11 (620I-X0177-G01) = 620I-X0136-G05种系化的+基因优化的

scFv+二硫化物稳定化

[0442] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVMSVMEALHNHYTQKSLSL
SPGKSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYVMHWVRQAPGKCLEWVSSIYPSGGLTKYADSVKGR
FTISRDNKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTMTVTVSSGGGGSGGGGSGGGGSGGGGS
DIVMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEIKR (SEQ ID NO:50)

[0443] 实施例4:具有C-末端突变和/或缺失的示例性双特异性抗体的构建和表征

[0444] 评估示例性双特异性抗体 (620I-X0177-A01、620I-X0177-C01、620I-X0177-E01、
620I-X0177-G01) 的生产性和可制造性。在分析前,将双特异性抗体的样品在室温,在各种
pH下孵育48小时。然后将样品在SDS-PAGE蛋白凝胶上分离,如例如针对图15中的双特异性
抗体620I-X0177-A01所示的,或通过尺寸排阻色谱分析(图16中的A-C)。在还原条件下观察到
30kDa和50kDa条带的pH依赖性增加和80kDa条带的降低(图15,泳道2-4)。这些意想不到的
条带的出现(appearance)表明双特异性抗体正在经历未预料到的蛋白水解裂解。在非还
原条件下,相同的30kDa种类的出现表明裂解的种类是单体的(图15,泳道6-8)。通过SEC分
析,在代表正确形成的双特异性抗体的15.7-16.1分钟,在代表DX-2930的17分钟,在代表裂
解的单链抗体的22分钟观察到峰(图16)。

[0445] 设计示例性双特异性抗体以去除IgG1重链C末端赖氨酸残基或将赖氨酸突变为甘
氨酸残基。

[0446] 以下提供双特异性抗体的第一多肽的氨基酸序列,所述第一多肽的第一抗体的重
链的C-末端赖氨酸残基缺失或C-末端赖氨酸突变为甘氨酸残基。这些第一多肽可以与DX-
2930的轻链配对。

[0447] >620I-X0179-A09 (IgG-C-末端赖氨酸缺失的620I-X0177-A01)

[0448] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVMSVMEALHNHYTQKSLSL
SPGSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSQYVMHWVRQAPGKCLEWVSSIWPSGGHTRYADSVKGRF
TISRDNKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTMTVTVSSGGGGSGGGGSGGGGSGGGGS
IVMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
LKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEIKR (SEQ ID NO:141)

[0449] >620I-X0179-C01 (IgG-C-末端赖氨酸突变为甘氨酸的620I-X0177-A01)

[0450] EVQLLESGGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF

TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGGSGGGSEVQLLES GGGVLVQPGGSLRLS CAASGFTFSQYVMHWVRQAPGKCLEWVSSIWPSSGGHTRYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTMTVTVSSGGGGSGGGSGGGSGGGGS
DIVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGCGTKVEIKR (SEQ ID NO:142)

[0451] >620I-X0179-E05 (IgG-C-末端赖氨酸缺失的620I-X0177-C01)

[0452] EVQLLES GGGVLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGGSGGGSEVQLLES GGGVLVQPGGSLRLS CAASGFTFSWYVMHWVRQAPGKCLEWVSSIYPSGGKTSYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTMTVTVSSGGGGSGGGSGGGSGGGGS
IVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
LKISRVEAEDVGYYCMQALQTPWTFGCGTKVEIKR (SEQ ID NO:143)

[0453] >620I-X0179-G05 (IgG-C-末端赖氨酸突变为甘氨酸的620I-X0177-C01)

[0454] EVQLLES GGGVLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGGSGGGSEVQLLES GGGVLVQPGGSLRLS CAASGFTFSWYVMHWVRQAPGKCLEWVSSIYPSGGKTSYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTMTVTVSSGGGGSGGGSGGGSGGGGS
DIVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGCGTKVEIKR (SEQ ID NO:144)

[0455] >620I-X0180-A05 (IgG-C-末端赖氨酸缺失的620I-X0177-G01)

[0456] EVQLLES GGGVLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGGSGGGSEVQLLES GGGVLVQPGGSLRLS CAASGFTFSHYVMHWVRQAPGKCLEWVSSIYPSGGLTKYADSVKGRF

TI SRD NSKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTTTVTVSSGGGGSGGGSGGGSGGGGS
IVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
LKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEIKR (SEQ ID NO:145)

[0457] >620I-X0180-C11 (IgG-C-末端赖氨酸突变为甘氨酸的620I-X0177-G01)

[0458] EVQLLES G GGLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TI SRD NSKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGGSGGGSEVQLLES G GGLVQPGGSLRLS CAASGFTFSHYVMHWVRQAPGKCLEWVSSIYPSGGLTKYADSVKGR
FTI SRD NSKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTTTVTVSSGGGGSGGGSGGGSGGGGS
DIVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEIKR (SEQ ID NO:146)

[0459] >620I-X0180-E07 (IgG-C-末端赖氨酸缺失的620I-X0177-E01)

[0460] EVQLLES G GGLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TI SRD NSKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGGSGGGSEVQLLES G GGLVQPGGSLRLS CAASGFTFSWYSMHVVRQAPGKCLEWVSVIYPSGGKTRYADSVKGRF
TI SRD NSKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTTTVTVSSGGGGSGGGSGGGSGGGGS
IVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
LKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEIKR (SEQ ID NO:147)

[0461] >620I-X0180-G03 (IgG-C-末端赖氨酸突变为甘氨酸的620I-X0177-E01)

[0462] EVQLLES G GGLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TI SRD NSKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGGSGGGSEVQLLES G GGLVQPGGSLRLS CAASGFTFSWYSMHVVRQAPGKCLEWVSVIYPSGGKTRYADSVKGR
FTI SRD NSKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTTTVTVSSGGGGSGGGSGGGSGGGGS
DIVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEIKR (SEQ ID NO:148)

[0463] 在 $t=0$ 时,通过在SDS-PAGE凝胶上分离双特异性抗体来评估每个示例性双特异性抗体,其第一抗体重链的C-末端赖氨酸残基缺失或C-末端赖氨酸突变为甘氨酸残基(图17)。每个示例性双特异性抗体的样品也使用Amicon 10kDa分子量截留旋转过滤器在50mM

Hepes, pH7.5中浓缩至约10mg/mL,并在室温孵育48小时。然后通过SDS-PAGE凝胶评估样品(图18)。在每种情况下,C-末端赖氨酸的缺失或突变降低或消除了来自双特异性抗体的scFv的裂解。

[0464] 双特异性的样品也通过分析尺寸排阻色谱进行评估,证明C-末端赖氨酸的缺失或突变降低了双特异性抗体的裂解(图19-20)。还通过将抗体与EndoLysC在37°C孵育1小时,然后在SDS-PAGE凝胶上分离,来评估双特异性抗体的裂解(图21)。50kDa的蛋白条带对应于DX-2930的Fab部分,和100kDa的条带对应于Fc-scFv的同源二聚体,进一步表明重链C-末端赖氨酸的缺失或突变降低了双特异性抗体的裂解。

[0465] 与DX-2930和DX-4012对照相比,C-末端赖氨酸缺失或突变的示例性双特异性抗体的特征还在于能够抑制pKa1、FXIIa和活化血浆(表1,图22-24)。发现每个示例性双特异性抗体在功能上等同于亲本双特异性抗体(该双特异性抗体不包含IgG1重链C-末端赖氨酸的缺失或突变)。突变可能降低双特异性抗体的电荷异质性。

[0466] 表6:用于生物化学测定的双特异性抗体以及DX-2930和DX-4012对照IgG的总结。进行测定以测量对pKa1的抑制、对FXIIa的抑制和对活化血浆的抑制。

[0467]

R-名称	X-名称(或 DX-)	亲本双特异性	IgG-C- 末端 改变	FXII scFv	PKa1 抑 制 $K_{i,app}$ (pM)	FXIIa 抑制 $K_{i,app}$ (pM)	活化血浆 抑制 IC50/ $K_{i,app}$ (pM)
620I-R0052-A01	620I-X0179-A09	620I-X0177-A01	Lys- 缺失	559C-M0192-H11 GL/GO/二硫化物 H4L scFv	225	90	7.6(Ki)
620I-R0052-C01	620I-X0179-C01	620I-X0177-A01	Lys-Gly	559C-M0192-H11 GL/GO/二硫化物 H4L scFv	243	108	7.8(Ki)
620I-R0052-E01	620I-X0179-E05	620I-X0177-C01	Lys- 缺失	559C-M0192-F06 GL/GO/二硫化物 H4L scFv	203	99	7.4(Ki)
620I-R0052-G01	620I-X0179-G05	620I-X0177-C01	Lys-Gly	559C-M0192-F06 GL/GO/二硫化物 H4L scFv	226	52	8.1(Ki)
620I-R0052-E03	620I-X0180-E07	620I-X0177-E01	Lys- 缺失	559C-M0191-E09 GL/GO/二硫化物 H4L scFv	243	92	3.0(Ki)
620I-R0052-G03	620I-X0180-G03	620I-X0177-E01	Lys-Gly	559C-M0191-E09 GL/GO/二硫化物 H4L scFv	208	71	2.8(Ki)

[0468]

620I-R0052-A03	620I-X0180-A05	620I-X0177-G01	Lys- 缺失	559C-M0192-A01 GL/GO/二硫化物 H4L scFv	254	116	7.3(Ki)
620I-R0052-C03	620I-X0180-C11	620I-X0177-G01	Lys-Gly	559C-M0192-A01 GL/GO/二硫化物 H4L scFv	232	140	9.2(Ki)
	DX-2930				127、 131、151	n/a	90、68、74、 74、 66(IC50)
	DX-4012				n/a	8、2、9	279、 291(IC50)

[0469] 可替代地或另外,在上述的抗FXIIa scFv的C-末端的Lys-Arg (KR) 基序可被去除。以下提供了示例性双特异性抗体多肽的氨基酸序列,所述多肽的第一抗体的重链的C-末端赖氨酸残基缺失或C-末端赖氨酸突变为甘氨酸残基并且scFv的C-末端赖氨酸-精氨酸残基缺失。

[0470] >620I-X0186-C05 (IgG-C-末端赖氨酸缺失和C-末端KR被去除的620I-X0177-A01)

[0471] EVQLLES GGGLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SPGSGGGSEVQLLES GGGLVQPGGSLRLS CAASGFTFSQYVMHWVRQAPGKCLEWVSSIWPSGGHTRYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGT TTVTVSSGGGGSGGGSGGGSGGGGSD
IVMTQSPLSLPVTPGEPASISCRSSQSLHSGNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
LKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEI (SEQ ID NO:151)

[0472] >620I-X0185-C01 (IgG-C-末端赖氨酸突变为甘氨酸和C-末端KR被去除的620I-X0177-A01) EVQLLES GGGLVQPGGSLRLS CAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKG
RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMVTVSSASTKGPSVFPLAPSSKSTSG
GTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDK
RVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKT
KPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHNHYTQKSLSL
SLSPGSGGGSEVQLLES GGGLVQPGGSLRLS CAASGFTFSQYVMHWVRQAPGKCLEWVSSIWPSGGHTRYADSVK
GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGT TTVTVSSGGGGSGGGSGGGSGGGGSD
GSDIVMTQSPLSLPVTPGEPASISCRSSQSLHSGNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGT
DFTLKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEI (SEQ ID NO:152)

[0473] >620I-X0186-E05 (IgG-C-末端赖氨酸缺失和C-末端KR被去除的620I-X0177-C01)

[0474] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGSGGGSEVQLLESGLLVQPGGSLRLSCAASGFTFSWYVMHWVRQAPGKCLEWVSSIYPSGGKTSYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTMTVTVSSGGGGSGGGSGGGSGGGGS
IVMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
LKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEI (SEQ ID NO:153)

[0475] >620I-X0185-E01 (IgG-C-末端赖氨酸突变为甘氨酸和C-末端KR被去除的620I-X0177-C01)

[0476] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGSGGGSEVQLLESGLLVQPGGSLRLSCAASGFTFSWYVMHWVRQAPGKCLEWVSSIYPSGGKTSYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTMTVTVSSGGGGSGGGSGGGSGGGGS
DIVMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
TLKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEI (SEQ ID NO:154)

[0477] >620I-X0186-A05 (IgG-C-末端赖氨酸缺失和C-末端KR被去除的620I-X0177-G01)

[0478] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV MHEALHNHYTQKSLSL
SPGSGGGSEVQLLESGLLVQPGGSLRLSCAASGFTFSHYVMHWVRQAPGKCLEWVSSIYPSGGTKYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTMTVTVSSGGGGSGGGSGGGSGGGGS
IVMTQSPLSLPVTPGEPASISCRSSQSLHNSGYNLYDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFT
LKISRVEAEDVGVYYCMQALQTPWTFGCGTKVEI (SEQ ID NO:155)

[0479] >620I-X0185-A03 (IgG-C-末端赖氨酸突变为甘氨酸和C-末端KR被去除的620I-X0177-G01)

[0480] EVQLLESGLLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSL
SPGGSGGGSEVQLLESGGGLVQPGGSLRLSCAASGFTFSHYVMHWVRQAPGKCLEWVSSIYPSGGLTKYADSVKGR
FTISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTITVTVSSGGGGSGGGGSGGGGSGGGGS
DIVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGCGTKVEI (SEQ ID NO:156)

[0481] >620I-X0186-G07 (IgG-C-末端赖氨酸缺失和C-末端KR被去除的620I-X0177-E01)

[0482] EVQLLES GGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSL
SPGGSGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSWYSMHVVRQAPGKCLEWVSVIYPSGGKTRYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCARQRYRGPKYYYYMDVWGQGTITVTVSSGGGGSGGGGSGGGGSGGGGS
IVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
LKISRVEAEDVGYYCMQALQTPWTFGCGTKVEI (SEQ ID NO:157)

[0483] >620I-X0185-G01 (IgG-C-末端赖氨酸突变为甘氨酸和C-末端KR被去除的620I-X0177-E01)

[0484] EVQLLES GGGLVQPGGSLRLSCAASGFTFSHYIMMWVRQAPGKGLEWVSGIYSSGGITVYADSVKGRF
TISRDN SKNTLYLQMNSLRAEDTAVYYCAYRRIGVPRRDEFDIWGQGTMTVTVSSASTKGPSVFPLAPSSKSTSGGT
AALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSVCSVMHEALHNHYTQKSLSL
SPGGSGGGSEVQLLES GGGLVQPGGSLRLSCAASGFTFSWYSMHVVRQAPGKCLEWVSVIYPSGGKTRYADSVKGR
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DIVMTQSPLSLPVTPGEPASISCRSSQSLLSNGYNYLDWYLQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDF
TLKISRVEAEDVGYYCMQALQTPWTFGCGTKVEI (SEQ ID NO:158)

[0485] 上面列出的多肽可以与DX-2930的轻链配对形成双特异性抗体,其也在本公开的范围之内。

[0486] 其他的实施方案

[0487] 本说明书中公开的所有特征可以以任何组合进行组合。本说明书中公开的每个特征可以由用于相同,等同或相似目的的可替代特征代替。因此,除非另有明确说明,否则所公开的每个特征仅仅是等同或类似特征的通用系列的实例。

[0488] 通过上面的描述,本领域技术人员可以容易地确定本发明的基本特征,并且在不脱离本发明的精神和范围的情况下,可以对本发明进行各种改变和修改以使其适应各种用途和条件。因此,其它实施方案也在权利要求书的范围内。

[0001]	序列表
[0002]	<110> 武田药品工业株式会社
[0003]	<120> 针对血浆激肽释放酶和因子XII的双特异性抗体
[0004]	<130> D0617.70065CN01
[0005]	<140> 尚未分配
[0006]	<141> 2015-12-31
[0007]	<150> US 62/261,609
[0008]	<151> 2015-12-01
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[0425]	Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser			
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[0457]	Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu			
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[0459]	Ser Leu Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr			
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[0855]	Ser Leu Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr		
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[2301]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[2303]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[2305]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[2307]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[2309]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser		

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[2313]	Ala Ser Gly Phe Thr Phe Ser Trp Tyr Val Met His Trp Val Arg Gln		
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[2317]	Gly Lys Thr Ser Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser		
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[2358]	Lys	Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Tyr
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[2398]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[2402]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[2404]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[2420]	Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Gln Gly Thr Thr Val		
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[2566]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[2601]	450 455 460
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[2606]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Ile Tyr Pro Ser Gly		
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[2608]	Gly Tyr Thr Arg Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser		
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[2630]	Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly		
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[2663]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[2667]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		
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[2669]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
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[2673]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
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[2675]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
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[2695]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[2697]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
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[2699]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[2703]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[2705]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly		
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[2707]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		
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[2709]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[2711]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[2713]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
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[2717]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr Ile		
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[2719]	Met Val Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser		
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[2770]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser	
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[2859]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn					
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[2865]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu					
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[2903]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[2905]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[2907]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
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[2915]	Arg Ile Val Pro Ser Gly Gly Met Thr Arg Tyr Ala Asp Ser Val Lys		
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[2980]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
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[3014]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser		
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[3016]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val		
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[3073]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		350
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[3075]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		365
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[3077]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[3079]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[3082]		420	425
[3083]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		430
[3084]		435	440
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[3086]	450	455	460
[3087]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[3089]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp		
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[3097]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[3140]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro			
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[3144]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr			
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[3146]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro			
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[3198]	Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
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[3203]	610 615 620
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[3208]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
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[3243]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro					
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[3247]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn					
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[3259]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr					
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[3279]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
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[3283]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp		
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[3285]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[3404]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
[3405]	675 680 685
[3406]	Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly
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[3408]	Gln Gly Thr Lys Val Glu Ile Lys Arg
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[3423]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val
[3424]	50 55 60
[3425]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
[3426]	65 70 75 80
[3427]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
[3428]	85 90 95
[3429]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp
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[3431]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
[3432]	115 120 125
[3433]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
[3434]	130 135 140
[3435]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
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[3437]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
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[3439]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[3441]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
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[3443]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser

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[3449]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
[3450]		260	265 270
[3451]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
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[3453]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
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[3455]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
[3456]	305	310	315 320
[3457]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
[3458]		325	330 335
[3459]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		
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[3461]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		
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[3463]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
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[3465]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[3467]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[3469]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[3471]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[3473]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
[3474]	450	455	460
[3475]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[3477]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp		
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[3479]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[3483]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		
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[3495]	Met Gln Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser			
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[3518]	Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val			
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[3520]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val			
[3521]		50	55	60
[3522]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr			
[3523]	65	70	75	80
[3524]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys			
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[3526]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp			
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[3532]	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr
[3533]	145					150					155					160
[3534]	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro
[3535]				165						170					175	
[3536]	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr
[3537]				180						185				190		
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[3541]		210						215					220			
[3542]	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu
[3543]	225					230					235					240
[3544]	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu
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[3581]	530 535 540
[3582]	Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Gln Arg Tyr Arg Gly
[3583]	545 550 555 560
[3584]	Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
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[3592]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
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[3595]	645 650 655
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[3631]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro			
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[3633]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr			
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[3635]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn			
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[3637]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser			
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[3639]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu			
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[3641]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu			
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[3645]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu			
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[3649]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn			
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[3651]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro			
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[3657]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
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[3659]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[3661]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[3665]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[3667]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
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[3669]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[3671]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp		
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[3673]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[3675]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly		
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[3677]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		
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[3679]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[3681]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[3683]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
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[3687]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Met Tyr Thr		
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[3689]	Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser		
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[3691]	Arg Ile Tyr Pro Ser Gly Gly Lys Thr Leu Tyr Ala Asp Ser Val Lys		
[3692]	645	650	655
[3693]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu		
[3694]	660	665	670
[3695]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		

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[3728]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
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[3730]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[3732]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		
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[3734]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		
[3735]	210	215	220
[3736]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
[3737]	225	230	235
			240

[3738]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[3740]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
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[3746]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
[3747]	305	310	315 320
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[3756]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[3758]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[3760]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[3770]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile Trp Pro Ser Gly		
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[3786]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
[3787]	625 630 635 640
[3788]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly
[3789]	645 650 655
[3790]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
[3791]	660 665 670
[3792]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
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[3794]	Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly
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[3819]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
[3820]	115 120 125
[3821]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr

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[3827]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[3859]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[3861]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
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[3863]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		

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[3871]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp			
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[3873]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe			
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[3904]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr			
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[3908]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val
[3909]	50 55 60
[3910]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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[3914]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp
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[3916]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
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[3919]	130 135 140
[3920]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
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[3922]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
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[3924]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[3929]	210 215 220
[3930]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
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[3932]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
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[3935]	260 265 270
[3936]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
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[3940]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
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[3946]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
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[3976]	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Gln	Met	Thr	Gln	Ser	Pro
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[3981]	625					630					635					640
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[4025]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
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[4027]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
[4028]	225 230 235 240
[4029]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
[4030]	245 250 255
[4031]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser

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[4033]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
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[4047]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[4057]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[4059]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp		
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[4061]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[4063]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly		
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[4065]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		
[4066]	530	535	540
[4067]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
[4068]	545	550	555
[4069]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
[4070]	565	570	575
[4071]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
[4072]	580	585	590
[4073]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser		

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[4077]	Met Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser		
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[4081]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu		
[4082]	660	665	670
[4083]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		
[4084]	675	680	685
[4085]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp		
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[4087]	Gly Lys Gly Thr Thr Val Thr Val Ser Ser		
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			160

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[4148]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[4150]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[4158]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Ile Tyr Pro Ser Gly
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[4178]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
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[4180]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
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[4202]	65	70	75
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[4204]	85	90	95
[4205]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp		
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[4211]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr		
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[4215]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[4217]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		
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[4219]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		
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[4225]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
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[4227]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
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[4229]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
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[4231]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
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[4233]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
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[4235]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		
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[4237]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		
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[4239]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
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[4241]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		

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[4247]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu			
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[4249]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser			
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[4251]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys			
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[4253]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp			
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[4255]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu			
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[4257]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly			
[4258]		515	520	525
[4259]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp			
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[4261]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe			
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[4263]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser			
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[4265]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu			
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[4267]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser			
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[4271]	Met Tyr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser			
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[4273]	Arg Ile Tyr Pro Ser Gly Gly Ile Thr His Tyr Ala Asp Ser Val Lys			
[4274]		645	650	655
[4275]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu			
[4276]		660	665	670
[4277]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr			
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[4279]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp			
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[4281]	Gly Lys Gly Thr Thr Val Thr Val Ser Ser			
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[4302]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp
[4303]	100 105 110
[4304]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
[4305]	115 120 125
[4306]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
[4307]	130 135 140
[4308]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
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[4310]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
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[4312]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[4314]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
[4315]	195 200 205
[4316]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
[4317]	210 215 220
[4318]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
[4319]	225 230 235 240
[4320]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
[4321]	245 250 255
[4322]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
[4323]	260 265 270
[4324]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
[4325]	275 280 285

[4326]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
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[4328]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
[4329]	305	310	315
[4330]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
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[4332]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		
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[4334]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		
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[4336]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
[4337]		370	375
[4338]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[4340]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[4342]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[4344]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[4346]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser		
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[4348]	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala		
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[4352]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Tyr Ile Ser Pro Ser Gly		
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[4354]	Gly Lys Thr Lys Tyr Thr Asp Ser Val Lys Gly Arg Phe Thr Ile Ser		
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[4356]	Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg		
[4357]		530	535
[4358]	Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Gln Arg Tyr Arg Gly		
[4359]		545	550
[4360]	Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val		
[4361]		565	570
[4362]	Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly		
[4363]		580	585
[4364]	Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro		
[4365]		595	600
[4366]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg		
[4367]		610	615

[4368]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
[4369]	625 630 635 640
[4370]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly
[4371]	645 650 655
[4372]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
[4373]	660 665 670
[4374]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
[4375]	675 680 685
[4376]	Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly
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[4389]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr
[4390]	20 25 30
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[4392]	35 40 45
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[4394]	50 55 60
[4395]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
[4396]	65 70 75 80
[4397]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
[4398]	85 90 95
[4399]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp
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[4401]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
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[4404]	130 135 140
[4405]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
[4406]	145 150 155 160
[4407]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
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[4409]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr

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[4417]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
[4418]	245	250	255
[4419]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
[4420]	260	265	270
[4421]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
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[4423]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
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[4425]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
[4426]	305	310	315
[4427]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
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[4432]	355	360	365
[4433]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
[4434]	370	375	380
[4435]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
[4436]	385	390	395
[4437]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
[4438]	405	410	415
[4439]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[4441]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
[4442]	435	440	445
[4443]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
[4444]	450	455	460
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[4449]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[4455]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
[4456]	545	550	555 560
[4457]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[4459]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
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[4461]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser		
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[4465]	Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser		
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[4467]	Tyr Ile Ser Pro Ser Gly Gly Lys Thr Lys Tyr Thr Asp Ser Val Lys		
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[4469]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu		
[4470]	660	665	670
[4471]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		
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[4473]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp		
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[4475]	Gly Lys Gly Thr Thr Val Thr Val Ser Ser		
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[4486]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr		
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[4490]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val		
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[4492]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr		
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[4516]	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser
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[4519]				275						280				285		
[4520]	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr
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[4538]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		430
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[4541]		450	455
[4542]	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala		460
[4543]		465	470
[4544]	Ala Ser Gly Phe Thr Phe Ser Arg Tyr Ile Met Gly Trp Val Arg Gln		475
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[4546]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile Tyr Pro Ser Gly		495
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[4548]	Gly Val Thr Arg Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser		510
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[4550]	Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg		525
[4551]		530	535
[4552]	Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Gln Arg Tyr Arg Gly		540
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[4554]	Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val		555
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[4558]	Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro		590
[4559]		595	600
[4560]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg		605
[4561]		610	615
[4562]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp		620
[4563]		625	630
[4564]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly		635
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[4566]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser		655
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[4568]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val		670
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[4588]	50 55 60
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[4595]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
[4596]	115 120 125
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[4598]	130 135 140
[4599]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
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[4601]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
[4602]	165 170 175
[4603]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[4605]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
[4606]	195 200 205
[4607]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
[4608]	210 215 220
[4609]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
[4610]	225 230 235 240
[4611]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
[4612]	245 250 255
[4613]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
[4614]	260 265 270
[4615]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
[4616]	275 280 285
[4617]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
[4618]	290 295 300
[4619]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn

[4620]	305	310	315	320
[4621]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro			
[4622]		325	330	335
[4623]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln			
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[4625]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val			
[4626]		355	360	365
[4627]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val			
[4628]		370	375	380
[4629]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro			
[4630]	385	390	395	400
[4631]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr			
[4632]		405	410	415
[4633]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val			
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[4635]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu			
[4636]		435	440	445
[4637]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser			
[4638]		450	455	460
[4639]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys			
[4640]	465	470	475	480
[4641]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp			
[4642]		485	490	495
[4643]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu			
[4644]		500	505	510
[4645]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly			
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[4647]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp			
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[4649]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe			
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[4651]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser			
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[4655]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser			
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[4657]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr Ile			
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[4659]	Met Gly Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser			
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[4661]	Ser Ile Tyr Pro Ser Gly Gly Val Thr Arg Tyr Ala Asp Ser Val Lys			

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[4665]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr					
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[4667]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp					
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[4680]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr					
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[4684]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val					
[4685]		50		55		60
[4686]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr					
[4687]	65		70		75	80
[4688]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys					
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[4690]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp					
[4691]		100		105		110
[4692]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro					
[4693]		115		120		125
[4694]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr					
[4695]		130		135		140
[4696]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr					
[4697]	145		150		155	160
[4698]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro					
[4699]		165		170		175
[4700]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr					
[4701]		180		185		190
[4702]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn					
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[4704]	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser
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[4707]	225					230					235				240	
[4708]	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu
[4709]					245					250					255	
[4710]	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser
[4711]				260					265					270		
[4712]	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu
[4713]		275						280					285			
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[4715]	290						295					300				
[4716]	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn
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[4723]		355						360					365			
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[4725]	370						375					380				
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[4735]	450					455						460				
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[4737]	465					470				475				480		
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[4746]	Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Gln Arg Tyr Arg Gly
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[4748]	Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
[4749]	565 570 575
[4750]	Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
[4751]	580 585 590
[4752]	Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
[4753]	595 600 605
[4754]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
[4755]	610 615 620
[4756]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
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[4758]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly
[4759]	645 650 655
[4760]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
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[4762]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
[4763]	675 680 685
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[4777]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr
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[4795]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
[4796]	165	170	175
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[4799]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		
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[4805]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[4815]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
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[4817]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		
[4818]	340	345	350
[4819]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		
[4820]	355	360	365
[4821]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
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[4823]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
[4824]	385	390	395
[4825]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
[4826]	405	410	415
[4827]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
[4828]	420	425	430
[4829]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		

[4830]	435	440	445
[4831]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
[4832]	450	455	460
[4833]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[4835]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp		
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[4837]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[4839]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly		
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[4841]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		
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[4843]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[4845]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[4847]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
[4848]	580	585	590
[4849]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser		
[4850]	595	600	605
[4851]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Tyr Ile		
[4852]	610	615	620
[4853]	Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser		
[4854]	625	630	635 640
[4855]	Ser Ile Tyr Pro Ser Gly Gly Val Thr Lys Tyr Ala Asp Ser Val Lys		
[4856]	645	650	655
[4857]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu		
[4858]	660	665	670
[4859]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		
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[4861]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp		
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[4874]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr
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[4879]	50 55 60
[4880]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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[4882]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
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[4896]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
[4897]	195 200 205
[4898]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
[4899]	210 215 220
[4900]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
[4901]	225 230 235 240
[4902]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
[4903]	245 250 255
[4904]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
[4905]	260 265 270
[4906]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
[4907]	275 280 285
[4908]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
[4909]	290 295 300
[4910]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
[4911]	305 310 315 320
[4912]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
[4913]	325 330 335

[4914]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		
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[4916]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		350
[4917]		355	360
[4918]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		365
[4919]		370	375
[4920]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		380
[4921]		385	390
[4922]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		395
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[4924]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		415
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[4926]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		430
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[4928]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser		445
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[4934]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Ile Tyr Pro Ser Gly		495
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[4953]		645	650
[4954]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser		655
[4955]		660	665
			670

[4956]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
[4957]	675 680 685
[4958]	Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly
[4959]	690 695 700
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[4974]	35 40 45
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[4976]	50 55 60
[4977]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
[4978]	65 70 75 80
[4979]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
[4980]	85 90 95
[4981]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp
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[4983]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
[4984]	115 120 125
[4985]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
[4986]	130 135 140
[4987]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
[4988]	145 150 155 160
[4989]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
[4990]	165 170 175
[4991]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
[4992]	180 185 190
[4993]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
[4994]	195 200 205
[4995]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
[4996]	210 215 220
[4997]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu

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[4999]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu			
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[5001]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser			
[5002]		260	265	270
[5003]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu			
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[5005]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr			
[5006]		290	295	300
[5007]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn			
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[5009]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro			
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[5011]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln			
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[5013]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val			
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[5015]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val			
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[5017]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro			
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[5025]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser			
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[5027]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys			
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[5037]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe			
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[5039]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser			

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[5045]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Phe Tyr Ser					
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[5047]	Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser					
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[5049]	Arg Ile Tyr Pro Ser Gly Gly Val Thr Lys Tyr Ala Asp Ser Val Lys					
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[5053]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr					
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[5055]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp					
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[5068]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr					
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[5074]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr					
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[5076]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys					
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[5078]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp					
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[5080]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro					
[5081]		115		120		125

[5082]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
[5083]	130 135 140
[5084]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
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[5086]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
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[5088]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[5093]	210 215 220
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[5095]	225 230 235 240
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[5097]	245 250 255
[5098]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
[5099]	260 265 270
[5100]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
[5101]	275 280 285
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[5103]	290 295 300
[5104]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
[5105]	305 310 315 320
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[5144]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp		
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[5150]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val		
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[5165]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr		

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[5171]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr		
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[5181]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr		
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[5207]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		

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[5213]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[5245]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu		
[5246]	660	665	670
[5247]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		
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[5249]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp		

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[5264]	Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val		
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[5266]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val		
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[5270]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys		
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[5276]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr		
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[5280]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
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[5282]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[5284]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		
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[5288]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
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[5336]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
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[5342]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
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[5344]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
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[5346]	Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly
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[5371]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro
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[5387]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu			
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[5389]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser			
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[5391]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu			
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[5393]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr			
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[5395]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn			
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[5421]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly					
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[5439]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu					
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[5441]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr					
[5442]		675		680		685
[5443]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp					
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[5482]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
[5483]	225 230 235 240
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[5498]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
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[5591]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
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[5595]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		
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[5607]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
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[5619]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[5621]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[5623]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
[5624]	580	585	590
[5625]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser		
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[5627]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Trp Tyr Val		

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[5634]	660	665	670
[5635]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		
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[5658]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys		
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[5672]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		190
[5673]		195	200
[5674]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		205
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[5677]		225	230
[5678]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		235
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[5680]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		255
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[5694]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		365
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[5698]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		395
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[5703]		435	440
[5704]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser		445
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[5706]	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala		460
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[5710]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile Tyr Pro Ser Gly		495
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			510

[5712]	Gly Asn Thr Lys Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
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[5752]	50 55 60
[5753]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

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[5757]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp			
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[5765]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro			
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[5767]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr			
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[5793]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro			
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[5870]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu	
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[5872]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu	
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[5874]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser	
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[5876]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu	
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[5878]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr	
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[5880]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
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[5884]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
[5885]	340 345 350
[5886]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
[5887]	355 360 365
[5888]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
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[5890]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
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[5896]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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[5913]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr
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[5915]	Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
[5916]	35 40 45
[5917]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val
[5918]	50 55 60
[5919]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
[5920]	65 70 75 80
[5921]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys

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[5925]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro					
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[5927]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr					
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[5929]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr					
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[5931]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro					
[5932]		165		170		175
[5933]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr					
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[5935]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn					
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[5937]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser					
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[5939]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu					
[5940]	225		230		235	240
[5941]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu					
[5942]		245		250		255
[5943]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser					
[5944]		260		265		270
[5945]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu					
[5946]		275		280		285
[5947]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr					
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[5949]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn					
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[5955]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val					
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[5957]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val					
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[5969]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[5973]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[5998]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr		
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[6000]	Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val		
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[6002]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val		
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[6004]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr		
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			80

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[6008]	Ala	Tyr	Arg	Arg	Ile	Gly	Val	Pro	Arg	Arg	Asp	Glu	Phe	Asp	Ile	Trp
[6009]				100					105					110		
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[6013]				130					135					140		
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[6015]	145					150					155				160	
[6016]	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro
[6017]				165						170					175	
[6018]	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr
[6019]				180						185				190		
[6020]	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn
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[6025]	225					230					235				240	
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[6058]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Ile Tyr Pro Ser Gly
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[6060]	Gly Ala Thr Val Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
[6061]	515 520 525
[6062]	Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
[6063]	530 535 540
[6064]	Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Gln Arg Tyr Arg Gly
[6065]	545 550 555 560
[6066]	Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val
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[6070]	Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
[6071]	595 600 605
[6072]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
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[6095]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr
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[6115]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[6121]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
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[6127]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
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[6147]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu			
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[6149]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser			
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[6151]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys			
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[6153]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp			
[6154]		485	490	495
[6155]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu			
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[6157]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly			
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[6159]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp			
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[6161]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe			
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[6163]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser			
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[6171]	Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser			
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[6173]	Arg Ile Tyr Pro Ser Gly Gly Ala Thr Val Tyr Ala Asp Ser Val Lys			

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[6175]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu					
[6176]		660		665		670
[6177]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr					
[6178]		675		680		685
[6179]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp					
[6180]		690		695		700
[6181]	Gly Lys Gly Thr Thr Val Thr Val Ser Ser					
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[6212]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr					
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[6215]		195		200		205

[6216]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		
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[6218]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
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[6220]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[6223]	260	265	270
[6224]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
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[6226]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
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[6228]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
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[6230]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
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[6234]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		
[6235]	355	360	365
[6236]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
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[6238]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[6240]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[6242]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[6267]	610 615 620
[6268]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
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[6295]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
[6296]	65 70 75 80
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[6298]	85 90 95
[6299]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp

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[6303]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr		
[6304]	130	135	140
[6305]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr		
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[6307]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
[6308]	165	170	175
[6309]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[6315]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
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[6317]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[6319]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
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[6321]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
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[6323]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
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[6325]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
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[6337]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[6355]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
[6356]	545	550	555 560
[6357]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[6365]	Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser		
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[6367]	Ser Ile Tyr Pro Ser Gly Gly Met Thr Lys Tyr Ala Asp Ser Val Lys		
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[6369]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu		
[6370]	660	665	670
[6371]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		
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[6373]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp		
[6374]	690	695	700
[6375]	Gly Lys Gly Thr Thr Val Thr Val Ser Ser		
[6376]	705	710	
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[6388]	Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
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[6392]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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[6406]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[6408]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
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[6410]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
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[6464]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly		635
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[6466]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser		655
[6467]		660	665
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[6468]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
[6469]	675 680 685
[6470]	Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly
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[6487]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val
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[6489]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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[6493]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp
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[6503]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[6505]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn
[6506]	195 200 205
[6507]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser
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[6529]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro			
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[6535]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu			
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[6549]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe			
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[6557]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Trp Tyr Ser					
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[6559]	Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser					
[6560]	625		630		635	640
[6561]	Val Ile Tyr Pro Ser Gly Gly Lys Thr Arg Tyr Ala Asp Ser Val Lys					
[6562]		645		650		655
[6563]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu					
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[6565]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr					
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[6586]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr					
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[6588]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys					
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[6590]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp					
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[6592]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro					
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[6598]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
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[6600]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[6656]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
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[6661]	660 665 670
[6662]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
[6663]	675 680 685
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[6665]	690 695 700
[6666]	Gln Gly Thr Lys Val Glu Ile Lys Arg
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[6677]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr

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[6679]	Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val		
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[6681]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val		
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[6683]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr		
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[6685]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys		
[6686]	85	90	95
[6687]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp		
[6688]	100	105	110
[6689]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro		
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[6695]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
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[6705]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[6707]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
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[6709]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
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[6711]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		
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[6715]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
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[6717]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		
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[6719]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		

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[6723]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[6848]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
[6849]	610 615 620
[6850]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
[6851]	625 630 635 640
[6852]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly
[6853]	645 650 655
[6854]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
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[6856]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
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[6875]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val
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[6881]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp
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[6885]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
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[6935]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp					
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[6937]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe					
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[6939]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser					
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[6941]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu					
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[6943]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser					
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[6945]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Trp Tyr Thr					
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[6951]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu					
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[6953]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr					
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[6955]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp					
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[6994]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
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[7046]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly
[7047]	645 650 655
[7048]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
[7049]	660 665 670
[7050]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
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[7109]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
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[7111]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[7113]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[7115]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[7117]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[7125]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[7131]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[7133]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[7135]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
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[7137]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser		
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[7139]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr Val		

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[7145]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu		
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[7147]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		
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[7149]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp		
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[7168]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr		
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[7170]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys		
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[7172]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp		
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[7174]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro		
[7175]	115	120	125
[7176]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr		
[7177]	130	135	140
[7178]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr		
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[7180]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
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[7182]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[7184]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		190
[7185]		195	200
[7186]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		205
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[7188]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		220
[7189]		225	230
[7190]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		235
[7191]		245	250
[7192]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		240
[7193]		260	265
[7194]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		255
[7195]		275	280
[7196]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr		285
[7197]		290	295
[7198]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		300
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[7203]		340	345
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[7212]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		415
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[7218]	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala		460
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[7220]	Ala Ser Gly Phe Thr Phe Ser Trp Tyr Val Met Gln Trp Val Arg Gln		475
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[7228]	Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Gln Arg Tyr Arg Gly
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[7233]	580 585 590
[7234]	Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
[7235]	595 600 605
[7236]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
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[7238]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
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[7240]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly
[7241]	645 650 655
[7242]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
[7243]	660 665 670
[7244]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
[7245]	675 680 685
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[7262]	35 40 45
[7263]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val
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[7265]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr

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[7269]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp			
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[7271]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro			
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[7273]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr			
[7274]		130	135	140
[7275]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr			
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[7277]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro			
[7278]		165	170	175
[7279]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr			
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[7281]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn			
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[7283]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser			
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[7285]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu			
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[7287]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu			
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[7289]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser			
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[7291]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu			
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[7301]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val			
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[7304]		370	375	380
[7305]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro			
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[7406]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val
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[7408]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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[7412]	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
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[7414]	Ala Ser Gly Phe Thr Phe Ser Gln Tyr Val Met His Trp Val Arg Gln
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[7416]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile Trp Pro Ser Gly
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[7418]	Gly Phe Thr Lys Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
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[7430]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
[7431]	610 615 620
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[7435]	645 650 655
[7436]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
[7437]	660 665 670
[7438]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
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[7469]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr
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[7471]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
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[7473]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[7475]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn

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[7481]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[7491]	Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro		
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[7505]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[7517]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		

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[7519]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[7521]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[7523]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
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[7525]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser		
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[7527]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gln Tyr Val		
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[7529]	Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser		
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[7558]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys		
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[7560]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp		
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[7566]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr		140
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[7568]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		155
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[7602]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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[7701]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser					
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[7703]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys					
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[7707]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu					
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[7713]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe					
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[7715]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser					
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[7717]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu					
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[7719]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser					
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[7727]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu					

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[7756]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro		
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[7762]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
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[7764]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[7776]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
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[7796]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu
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[7798]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser
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[7805]	500 505 510
[7806]	Gly Met Thr Gln Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
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[7818]	Leu	Ser	Leu	Pro	Val	Thr	Pro	Gly	Glu	Pro	Ala	Ser	Ile	Ser	Cys	Arg
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[7851]	Ala	Tyr	Arg	Arg	Ile	Gly	Val	Pro	Arg	Arg	Asp	Glu	Phe	Asp	Ile	Trp
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[7859]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
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[7861]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		175
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[7867]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
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[7869]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[7871]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		255
[7872]	260	265	270
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[7877]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
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[7880]		325	330
[7881]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		335
[7882]	340	345	350
[7883]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		
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[7885]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
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[7887]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[7895]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		

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[7907]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[7909]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[7911]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
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[7919]	Arg Ile Tyr Pro Ser Gly Gly Met Thr Gln Tyr Ala Asp Ser Val Lys		
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[7959]				180						185					190	
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[8269]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln		
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[8271]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		
[8272]	355	360	365
[8273]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		

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[8275]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[8277]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[8279]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[8281]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[8283]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
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[8285]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[8287]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp		
[8288]		485 490	495
[8289]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[8291]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly		
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[8293]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		
[8294]		530 535	540
[8295]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[8297]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[8299]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
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[8301]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser		
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[8303]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gln Tyr Val		
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[8305]	Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser		
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[8307]	Arg Ile Tyr Pro Ser Gly Gly Val Thr Lys Tyr Ala Asp Ser Val Lys		
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[8309]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu		
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[8311]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		
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[8334]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys	
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[8336]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp	
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[8338]	Gly Gln Gly Thr Met Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro	
[8339]	115 120 125	
[8340]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr	
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[8345]	165 170 175	
[8346]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr	
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[8348]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn	
[8349]	195 200 205	
[8350]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser	
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[8354]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu	
[8355]	245 250 255	
[8356]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser	
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[8374]	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr
[8375]						405					410					415
[8376]	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val
[8377]						420					425					430
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[8403]	625 630 635 640
[8404]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly
[8405]	645 650 655
[8406]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
[8407]	660 665 670
[8408]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
[8409]	675 680 685
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[8445]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn					
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[8483]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu					

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[8487]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		
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[8489]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[8491]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		
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[8493]	Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Glu		
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[8495]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser		
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[8501]	Arg Ile Trp Pro Ser Gly Gly Lys Thr Thr Tyr Ala Asp Ser Val Lys		
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[8503]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu		
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[8505]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr		
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[8507]	Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp		
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[8552]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
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[8554]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
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[8562]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
[8563]	355 360 365
[8564]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
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[8566]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
[8567]	385 390 395 400

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[8570]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[8600]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser		
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[8623]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr	
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[8641]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser	
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[8649]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu	
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[8651]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr	

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[8659]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		350
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[8661]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		365
[8662]	370	375	380
[8663]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[8665]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[8667]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		415
[8668]		420	425
[8669]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		430
[8670]	435	440	445
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[8673]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[8675]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp		
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[8677]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		495
[8678]		500	505
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[8681]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		
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[8683]	Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe		
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[8685]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser		560
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[8724]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp			
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[8730]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr			
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[8742]	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu
[8743]						245										255
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[8788]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
[8789]	610 615 620
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[8865]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
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[8867]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[8962]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser		
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[8966]	Ala Ser Gly Phe Thr Phe Ser Gln Tyr Val Met His Trp Val Arg Gln		
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[8970]	Gly His Thr Arg Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser		
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[9015]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp
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[9025]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr
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[9053]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[9055]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[9057]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
[9058]	435	440	445
[9059]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
[9060]	450	455	460
[9061]	Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys		
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[9063]	Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp		
[9064]	485	490	495
[9065]	Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu		
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[9067]	Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly		
[9068]	515	520	525
[9069]	Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp		
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[9073]	Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Gly Gly Gly Gly Ser			
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[9077]	Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly Ser			
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[9079]	Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Gln Tyr Val			
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[9081]	Met His Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val Ser			
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[9083]	Ser Ile Trp Pro Ser Gly Gly His Thr Arg Tyr Ala Asp Ser Val Lys			
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[9102]	Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser His Tyr			
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[9104]	Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val			
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[9106]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val			
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[9108]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr			
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[9110]	Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys			
[9111]		85	90	95
[9112]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp			
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[9124]	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn
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[9126]	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser
[9127]		210					215					220				
[9128]	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu
[9129]	225					230				235					240	
[9130]	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu
[9131]				245					250					255		
[9132]	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser
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[9143]			340						345					350		
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[9145]		355					360					365				
[9146]	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val
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[9149]	385					390				395						400
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[9153]			420						425					430		
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[9155]			435					440					445			

[9156]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser
[9157]	450 455 460
[9158]	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala
[9159]	465 470 475 480
[9160]	Ala Ser Gly Phe Thr Phe Ser Asn Tyr Val Met His Trp Val Arg Gln
[9161]	485 490 495
[9162]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Ser Ile Trp Pro Ser Gly
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[9164]	Gly Lys Thr Lys Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser
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[9166]	Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg
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[9168]	Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Gln Arg Tyr Arg Gly
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[9170]	Pro Lys Tyr Tyr Tyr Tyr Met Asp Ala Trp Gly Gln Gly Thr Thr Val
[9171]	565 570 575
[9172]	Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
[9173]	580 585 590
[9174]	Gly Gly Ser Gly Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser Pro
[9175]	595 600 605
[9176]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
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[9178]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
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[9201]	Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val			
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[9203]	Ser Gly Ile Tyr Ser Ser Gly Gly Ile Thr Val Tyr Ala Asp Ser Val			
[9204]	50	55	60	
[9205]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr			
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[9217]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro			
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[9219]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr			
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[9250]	420							425					430				
[9251]	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	
[9252]	435							440					445				
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[9298]	Ile Met Met Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val		
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[9310]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr		
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[9312]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr		
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[9314]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
[9315]	165	170	175
[9316]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[9318]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		
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[9320]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		
[9321]	210	215	220
[9322]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
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			240

[9324]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[9332]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn		
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[9338]	Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val		
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[9348]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[9350]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser		
[9351]		450	455 460
[9352]	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala		
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[9354]	Ala Ser Gly Phe Thr Phe Ser Phe Tyr Ser Met His Trp Val Arg Gln		
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[9356]	Ala Pro Gly Lys Gly Leu Glu Trp Val Ser Arg Ile Tyr Pro Ser Gly		
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[9358]	Gly Ile Thr Ser Tyr Ala Asp Ser Val Lys Gly Arg Phe Thr Ile Ser		
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[9362]	Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr Arg Gln Arg Tyr Arg Gly		
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[9364]	Pro Lys Tyr Tyr Tyr Tyr Met Asp Val Trp Gly Lys Gly Thr Thr Val		
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[9369]	595 600 605
[9370]	Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg
[9371]	610 615 620
[9372]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp
[9373]	625 630 635 640
[9374]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly
[9375]	645 650 655
[9376]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser
[9377]	660 665 670
[9378]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val
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[9381]	690 695 700
[9382]	Gln Gly Thr Lys Val Glu Ile Lys Arg
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[9415]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		
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[9417]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		
[9418]	210	215	220
[9419]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
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[9421]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[9423]	Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser		
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[9425]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu		
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[9445]	Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu		
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[9447]	Ser Pro Gly Lys Ser Gly Gly Gly Ser Asp Ile Gln Met Thr Gln Ser		
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[9473]	Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu			
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[9475]	Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Thr			
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[9496]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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[9500]	Ala Arg Gln Arg Tyr Arg Gly Pro Lys Tyr Tyr Tyr Tyr Met Asp Val
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[9629]	35 40 45
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[9631]	50 55 60
[9632]	Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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[9637]	100 105 110
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[9654]	Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
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[10032]	Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser
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[10186]	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr
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[10212]	Pro Gly Lys Cys Leu Glu Trp Val Ser Ser Ile Tyr Pro Ser Gly Gly
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			240

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[11072]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp		
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[11076]	Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr		
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[11078]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr		
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[11084]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		
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[11086]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		
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[11128]	Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gln Arg Tyr Arg Gly Pro		
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[11132]	Val Ser Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
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[11134]	Gly Ser Gly Gly Gly Gly Ser Asp Ile Val Met Thr Gln Ser Pro Leu
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[11136]	Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser
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[11138]	Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr
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[11146]	Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly Cys
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[11165]	Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
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[11169]	Ala Tyr Arg Arg Ile Gly Val Pro Arg Arg Asp Glu Phe Asp Ile Trp
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[11175]	Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr		
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[11177]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro		
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[11179]	Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr		
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[11181]	Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn		
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[11183]	His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser		
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[11185]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu		
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[11187]	Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu		
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[11203]	Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val		
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[11205]	Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro		
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[11207]	Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr		
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[11209]	Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val		
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[11219]	Ala Pro Gly Lys Cys Leu Glu Trp Val Ser Ser Ile Tyr Pro Ser Gly		495
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[11236]	625	630	635
[11237]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly		640
[11238]		645	650
[11239]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser		655
[11240]		660	665
[11241]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val		670
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[11243]	Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly		685
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[11283]		225				230				235				240		
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[11285]			245						250					255		
[11286]	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser
[11287]			260						265					270		
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[11308]	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu
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[11313]				465					470					475		480
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[11371]	Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro
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[11378]	210 215 220
[11379]	Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
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[11385]	His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu					
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[11387]	Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr					
[11388]		290		295		300
[11389]	Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn					
[11390]	305		310		315	320
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[11393]	Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln					
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[11407]	Ser Pro Gly Gly Ser Gly Gly Gly Ser Glu Val Gln Leu Leu Glu Ser					
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[11409]	Gly Gly Gly Leu Val Gln Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala					
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[11411]	Ala Ser Gly Phe Thr Phe Ser Trp Tyr Ser Met His Trp Val Arg Gln					
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[11413]	Ala Pro Gly Lys Cys Leu Glu Trp Val Ser Val Ile Tyr Pro Ser Gly					
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[11417]	Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg					
[11418]		530		535		540
[11419]	Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Gln Arg Tyr Arg Gly					
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[11429]	Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr Asn Tyr Leu Asp Trp					
[11430]	625		630		635	640
[11431]	Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Leu Gly					
[11432]		645		650		655
[11433]	Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser					
[11434]		660		665		670
[11435]	Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val					
[11436]		675		680		685
[11437]	Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr Pro Trp Thr Phe Gly					
[11438]		690		695		700
[11439]	Cys Gly Thr Lys Val Glu Ile					
[11440]	705		710			

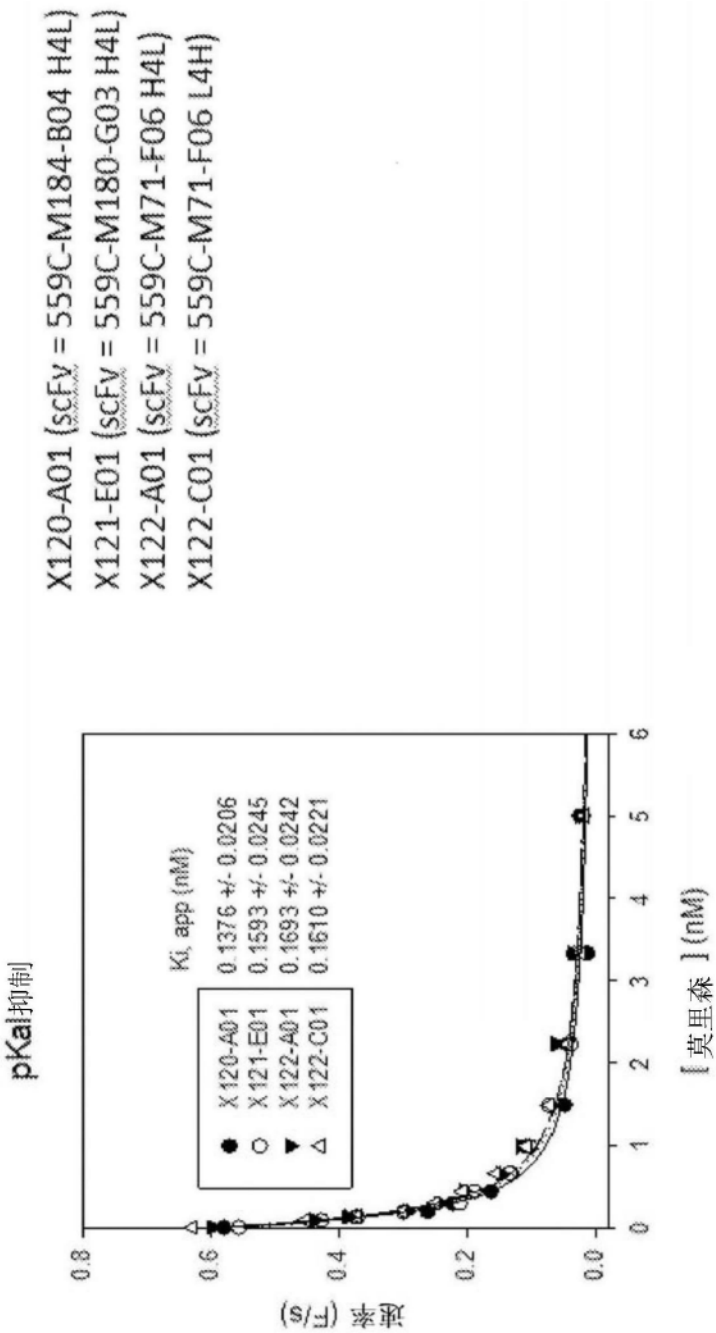


图1

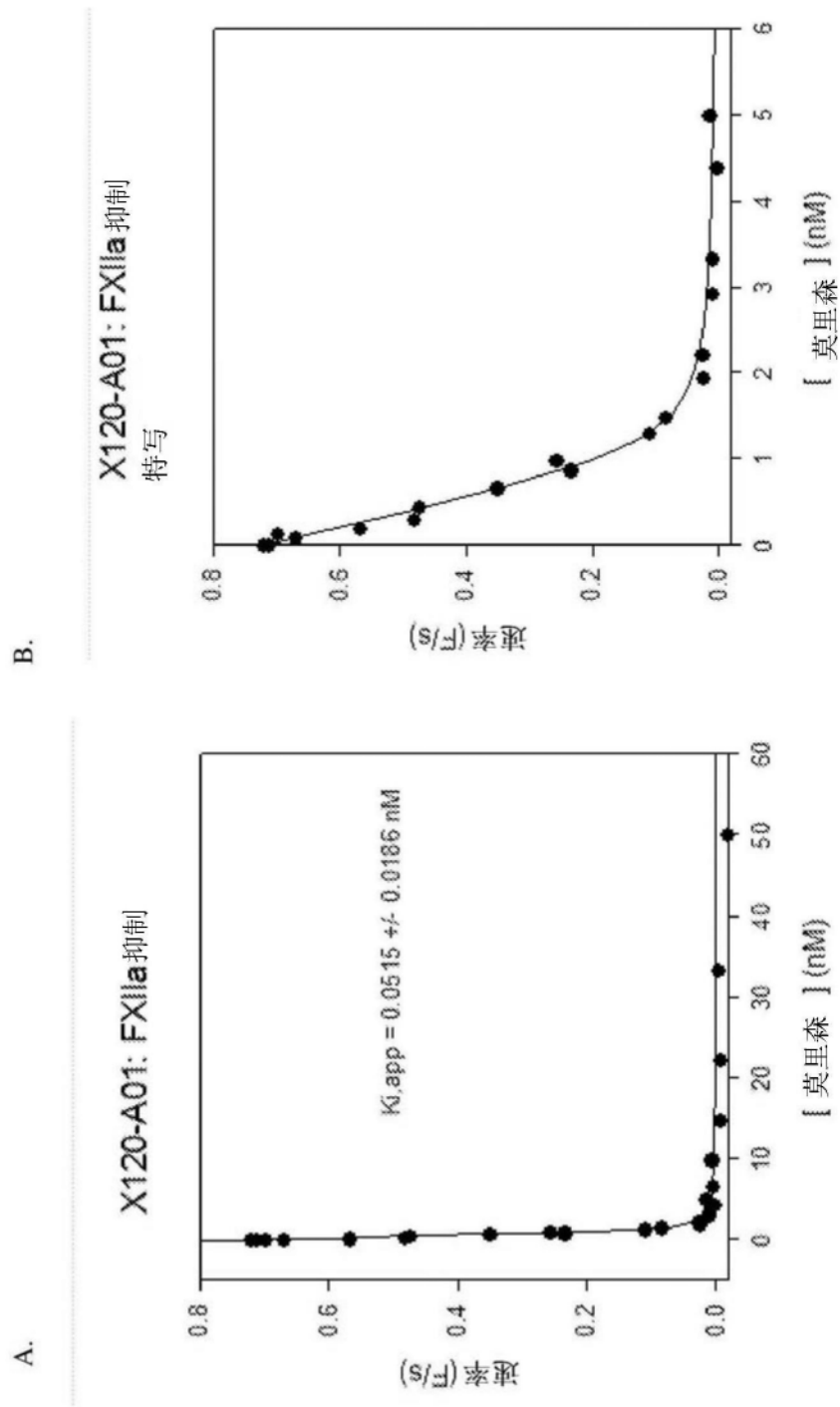


图2

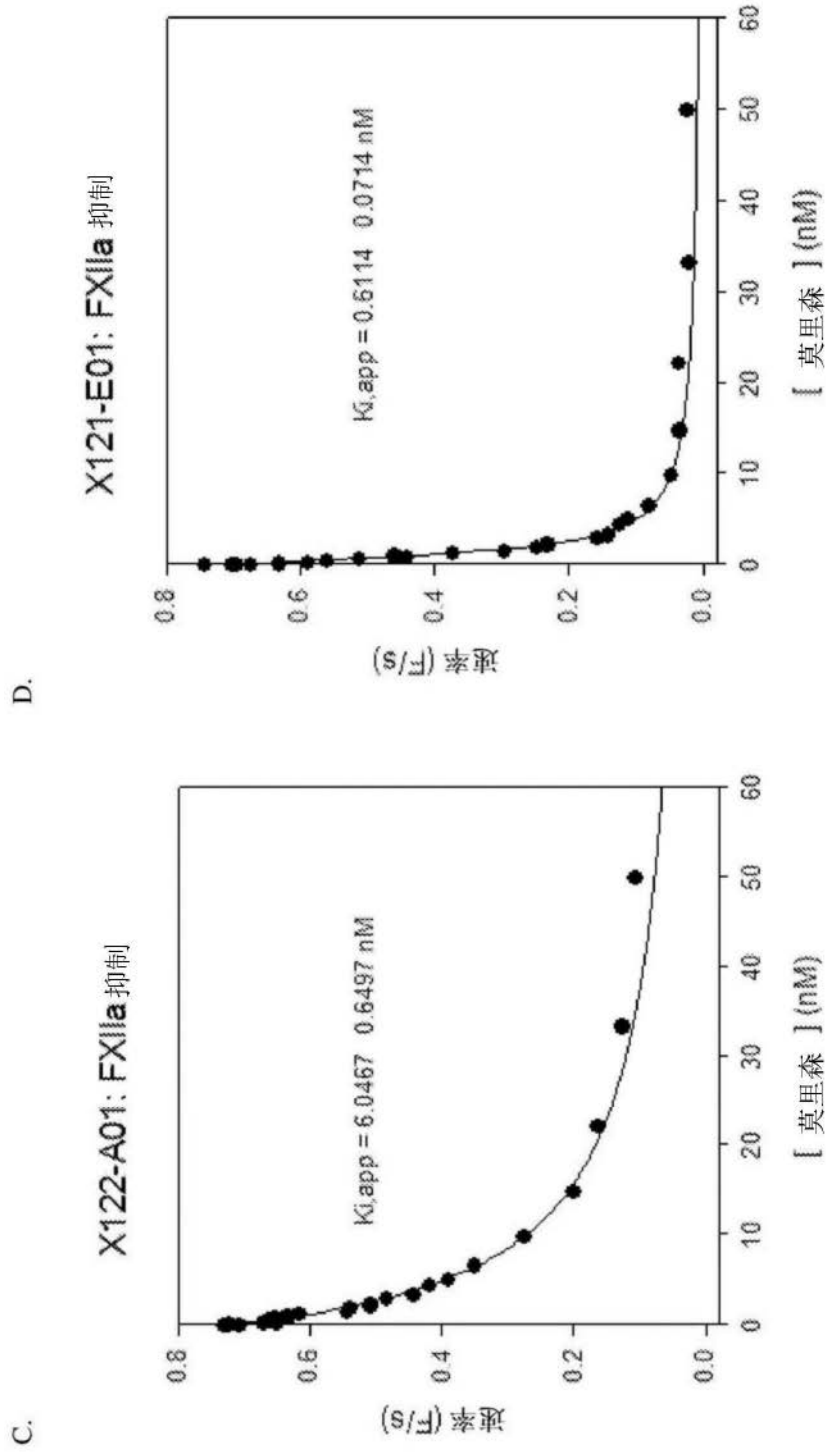
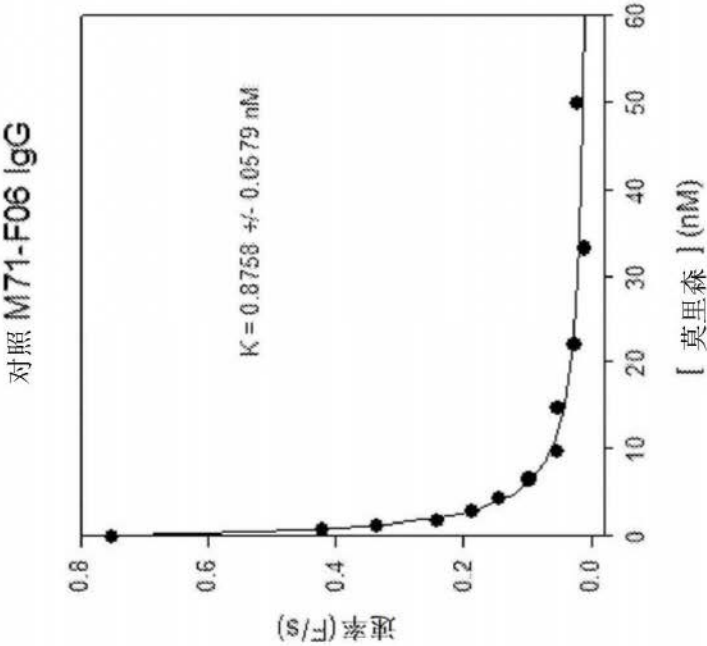


图2 (续)

F.



E.

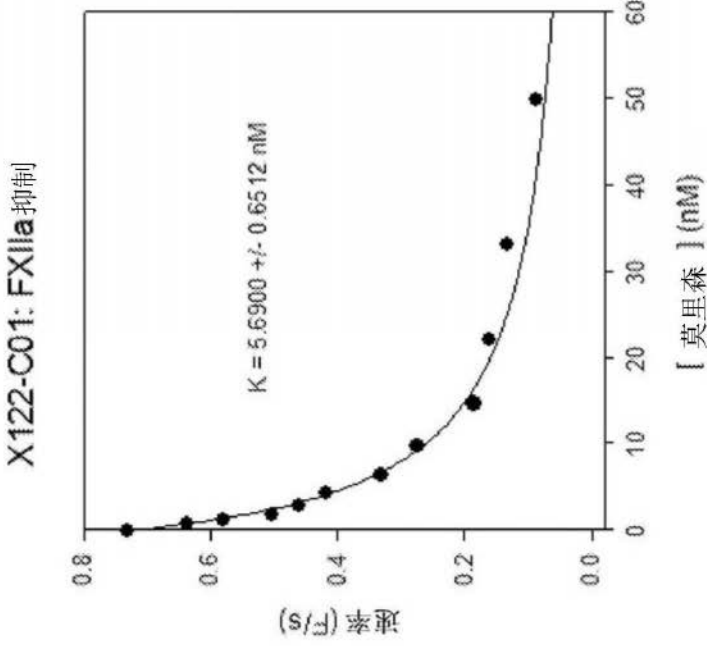
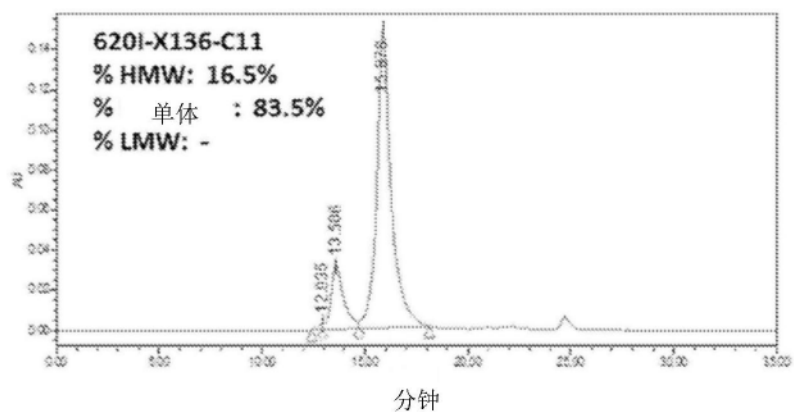
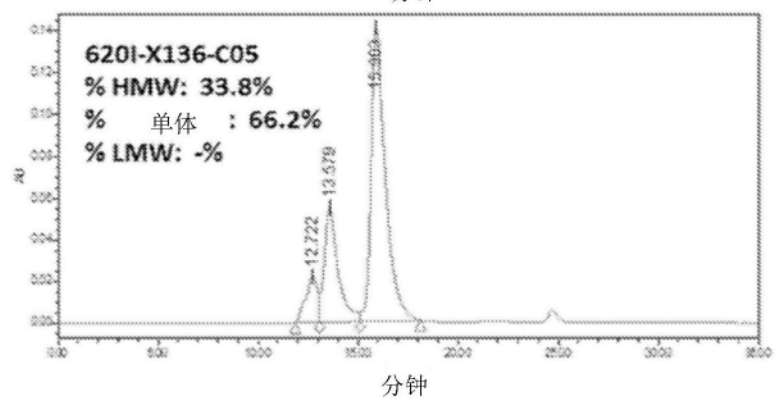


图2 (续)

A.



B.



C.

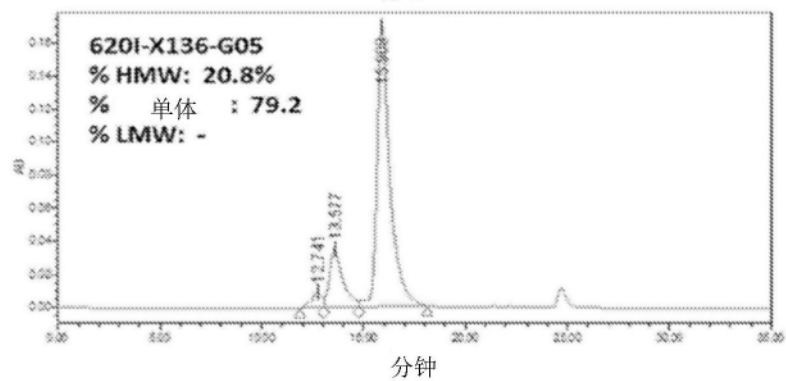
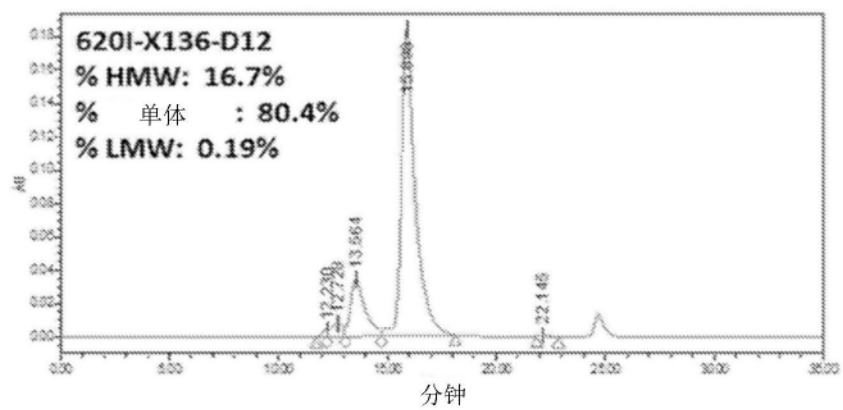


图3

D.



E.

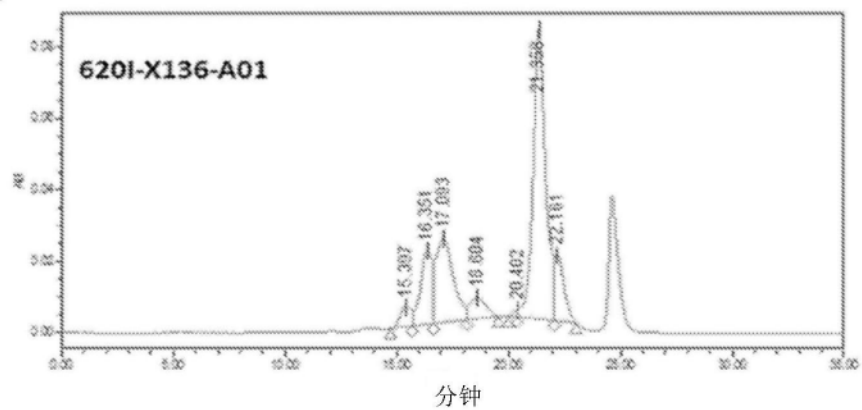


图3(续)

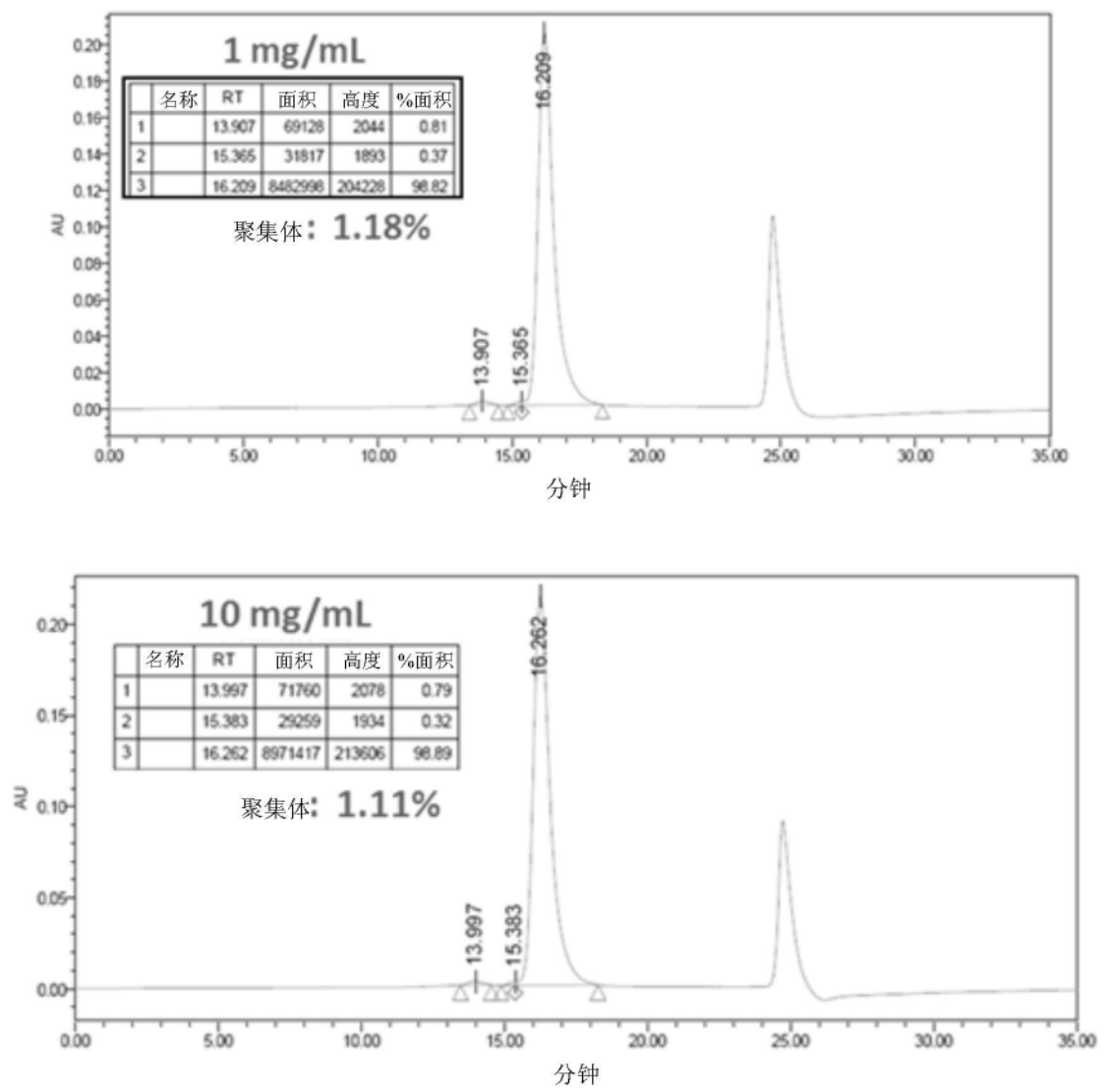


图4

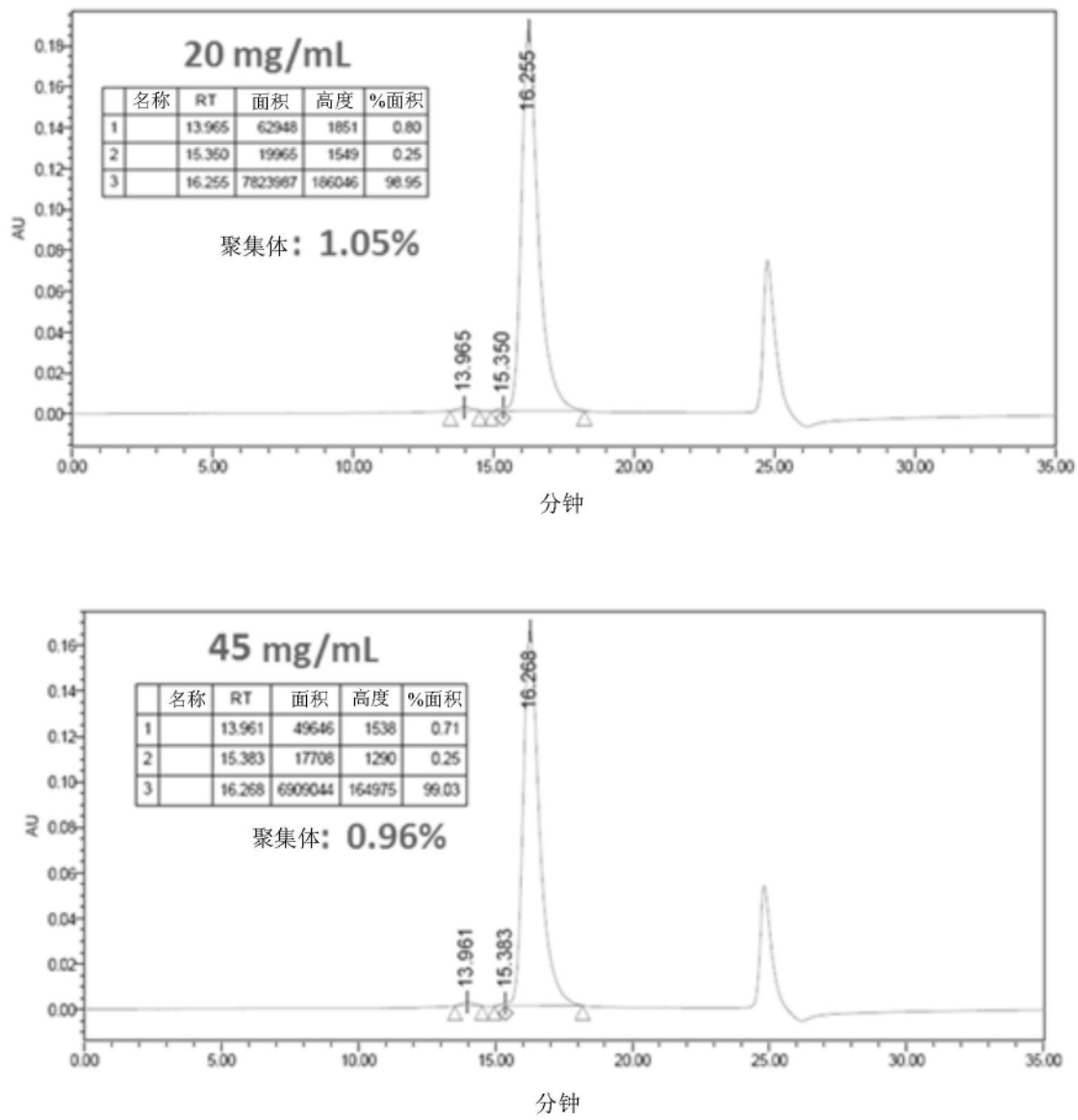


图4(续)

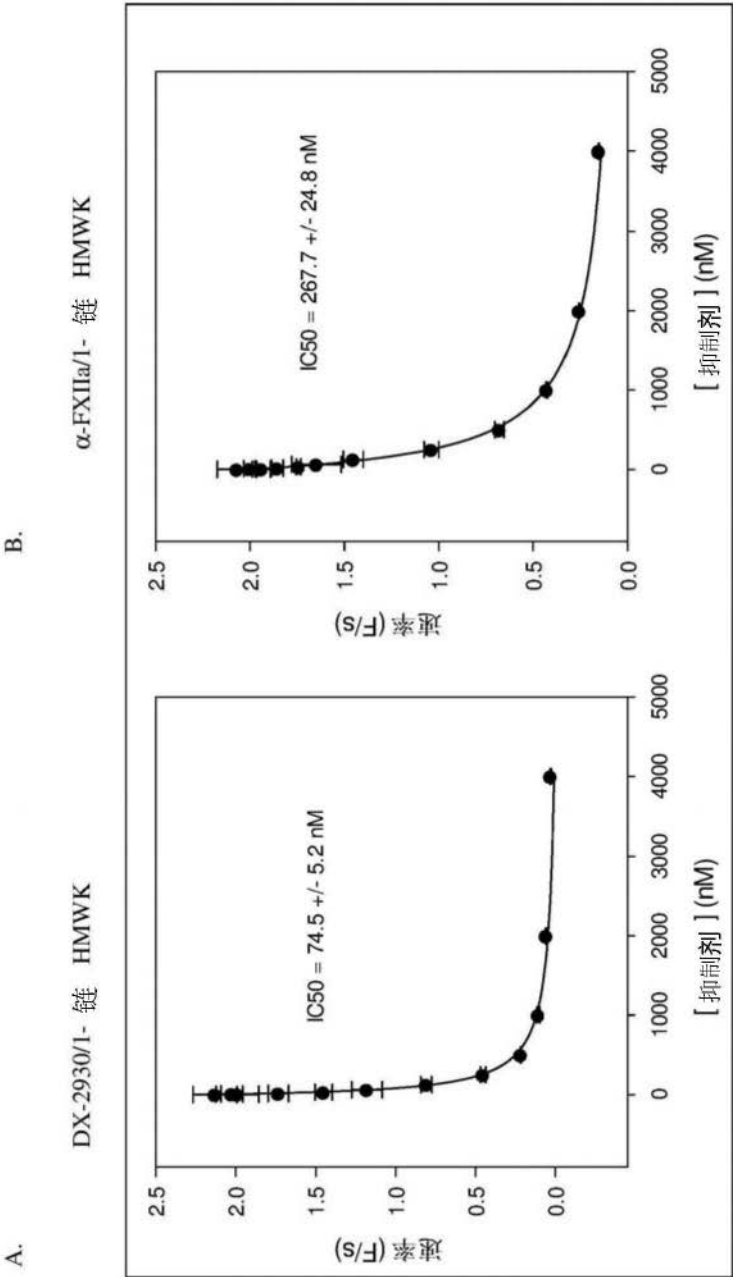


图5

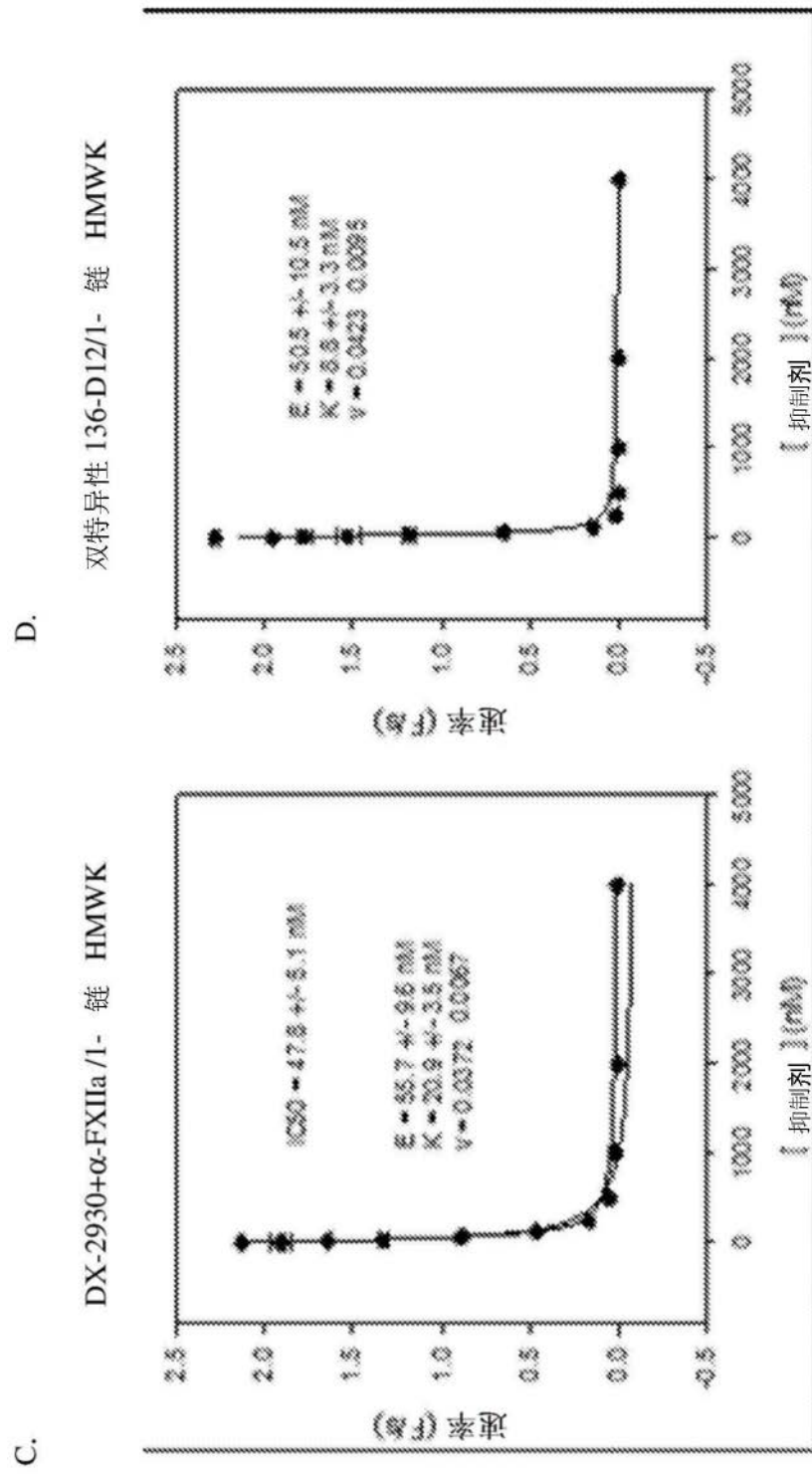


图5(续)

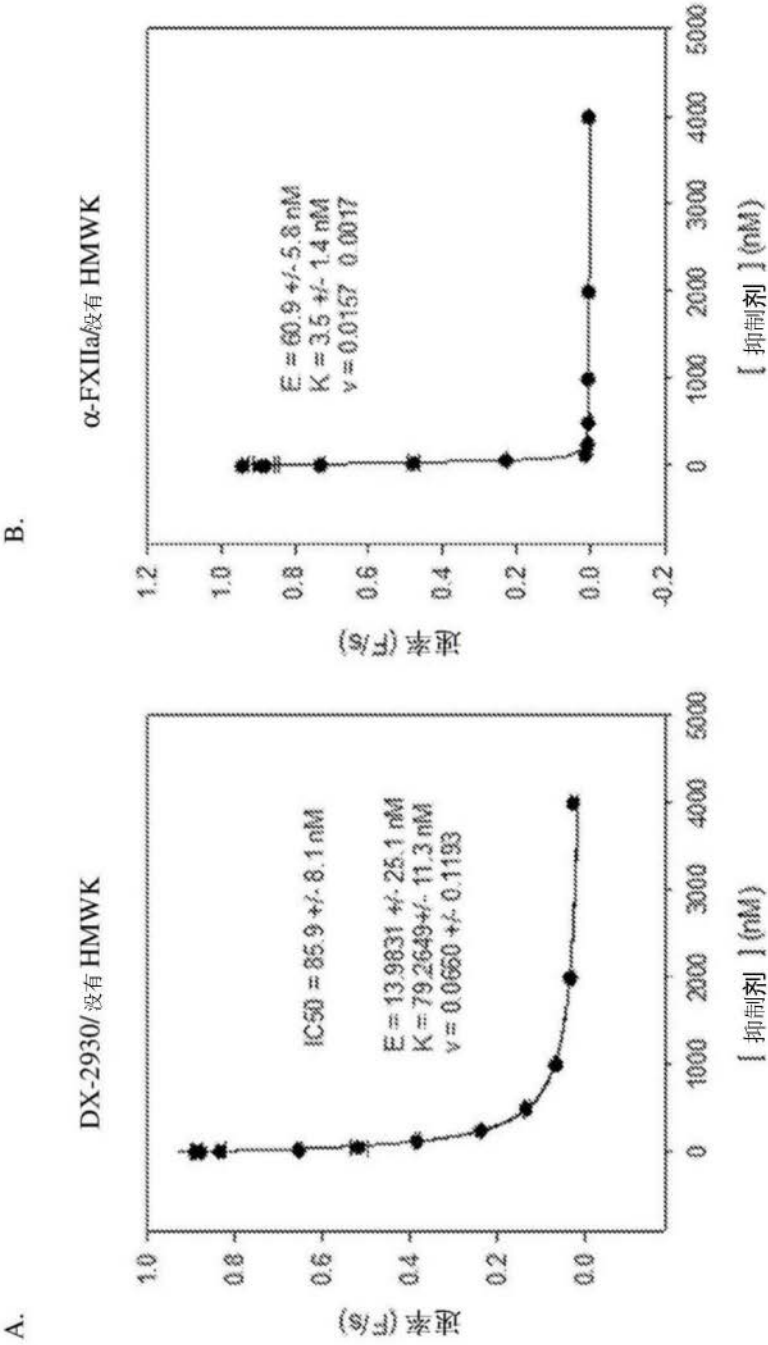


图6

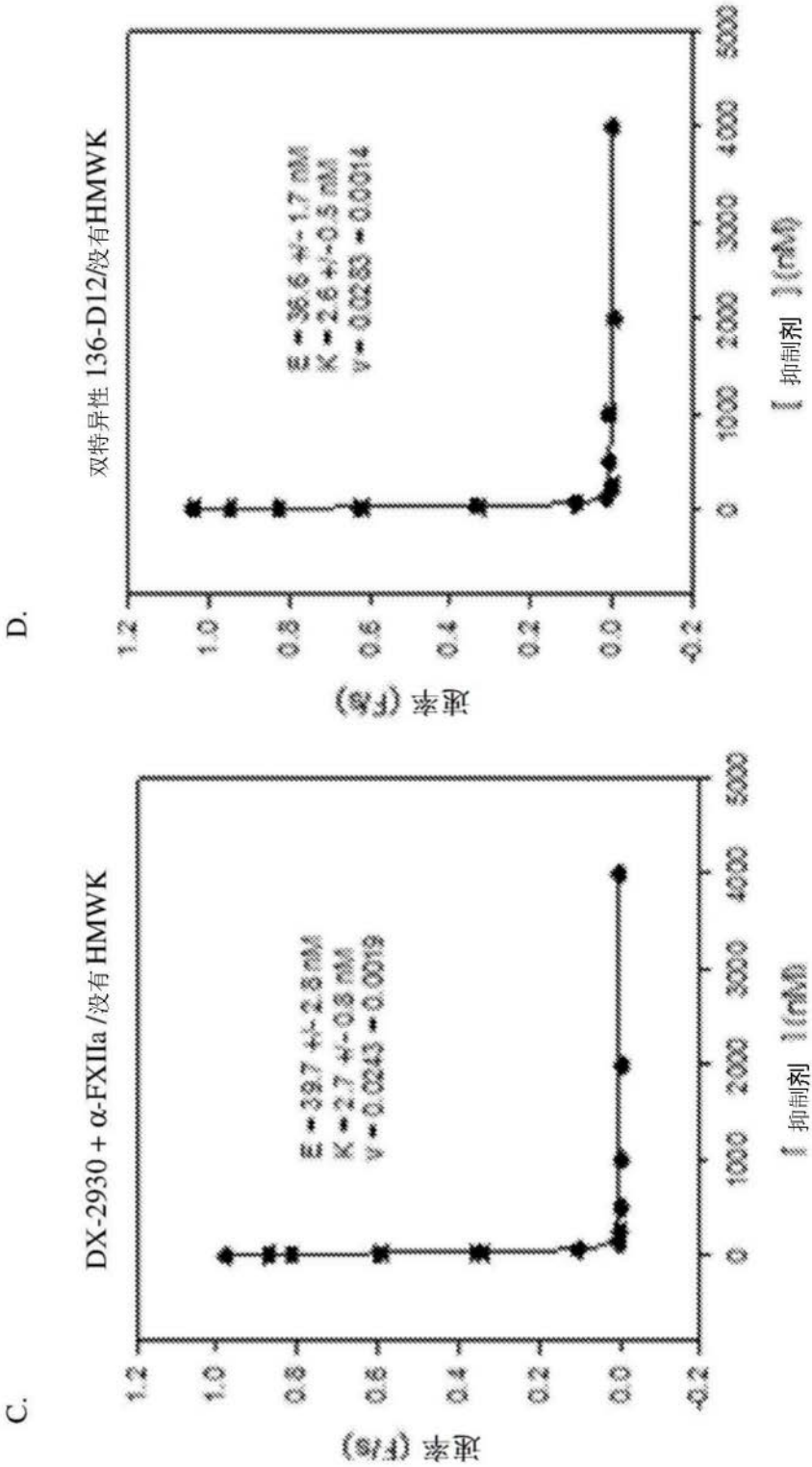


图6(续)

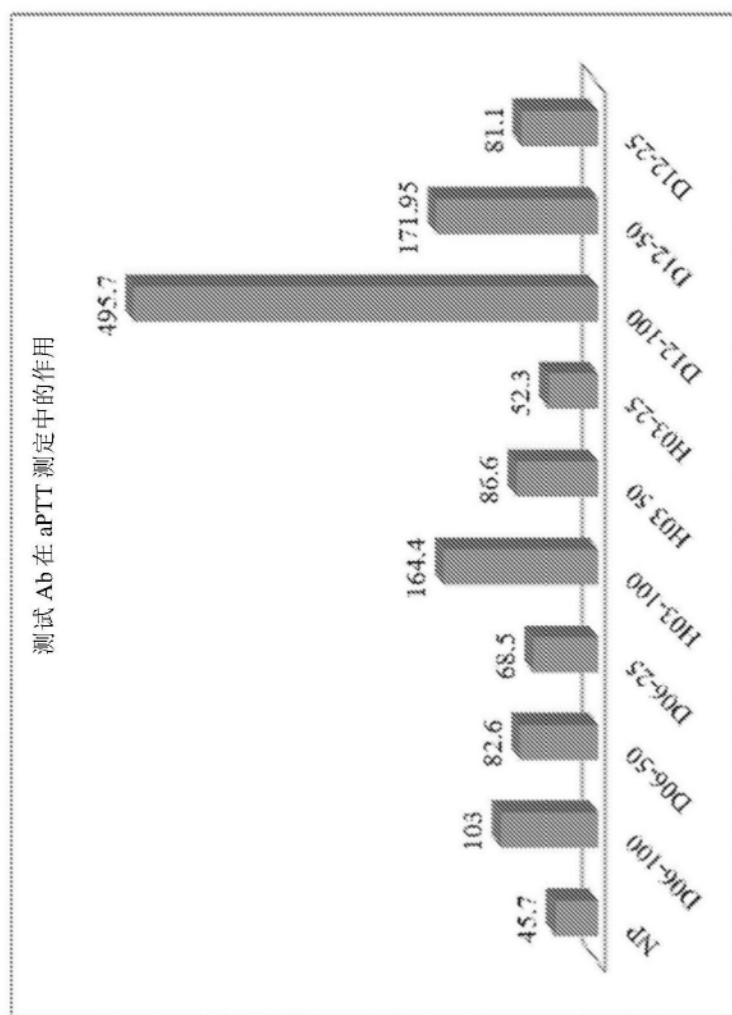


图7

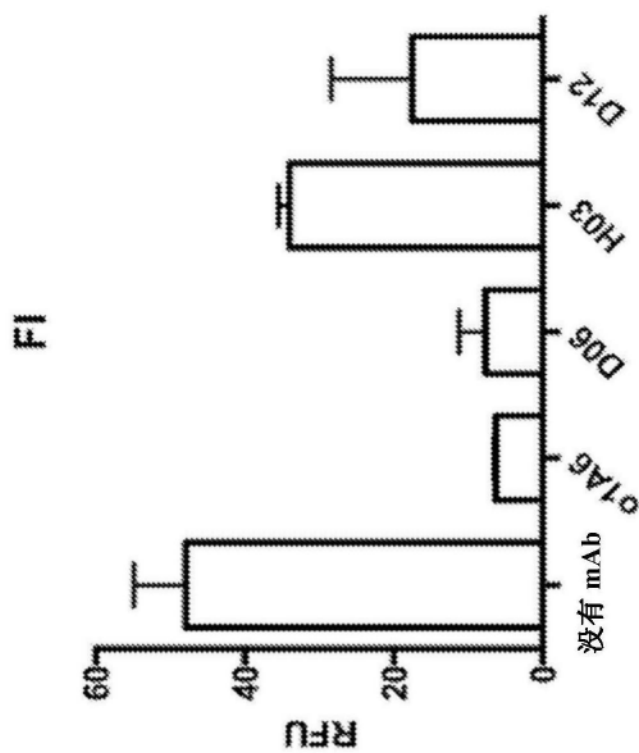


图8

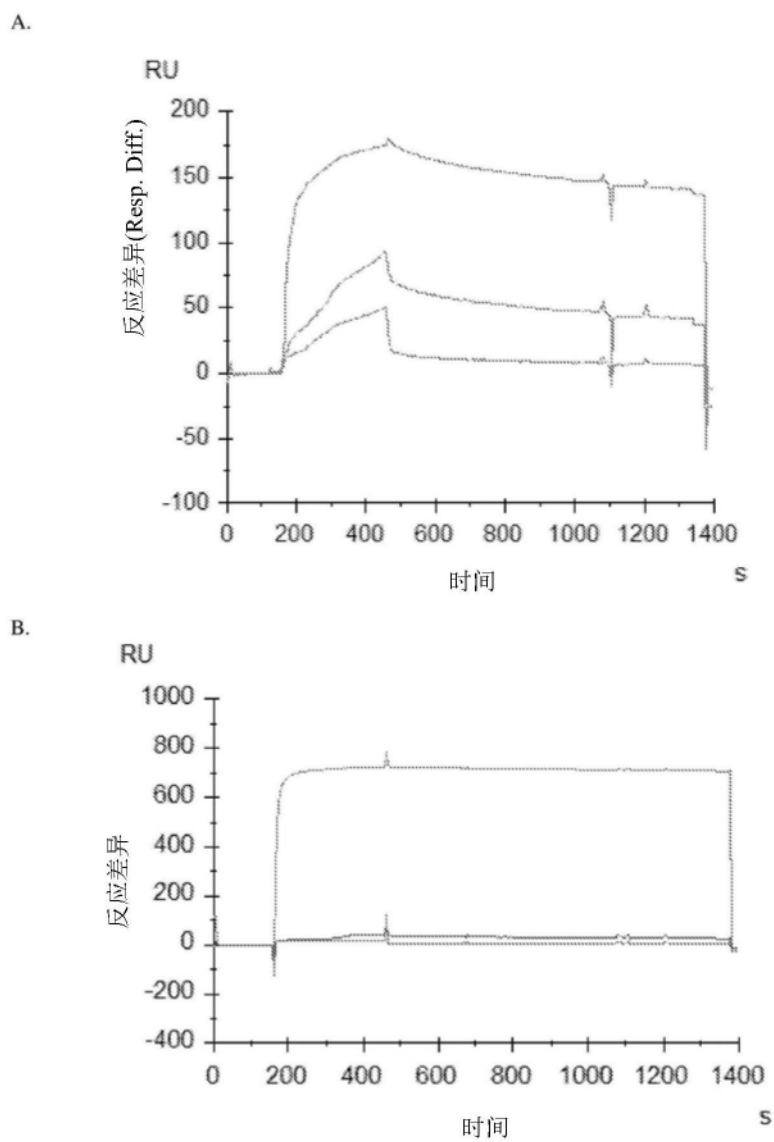


图9

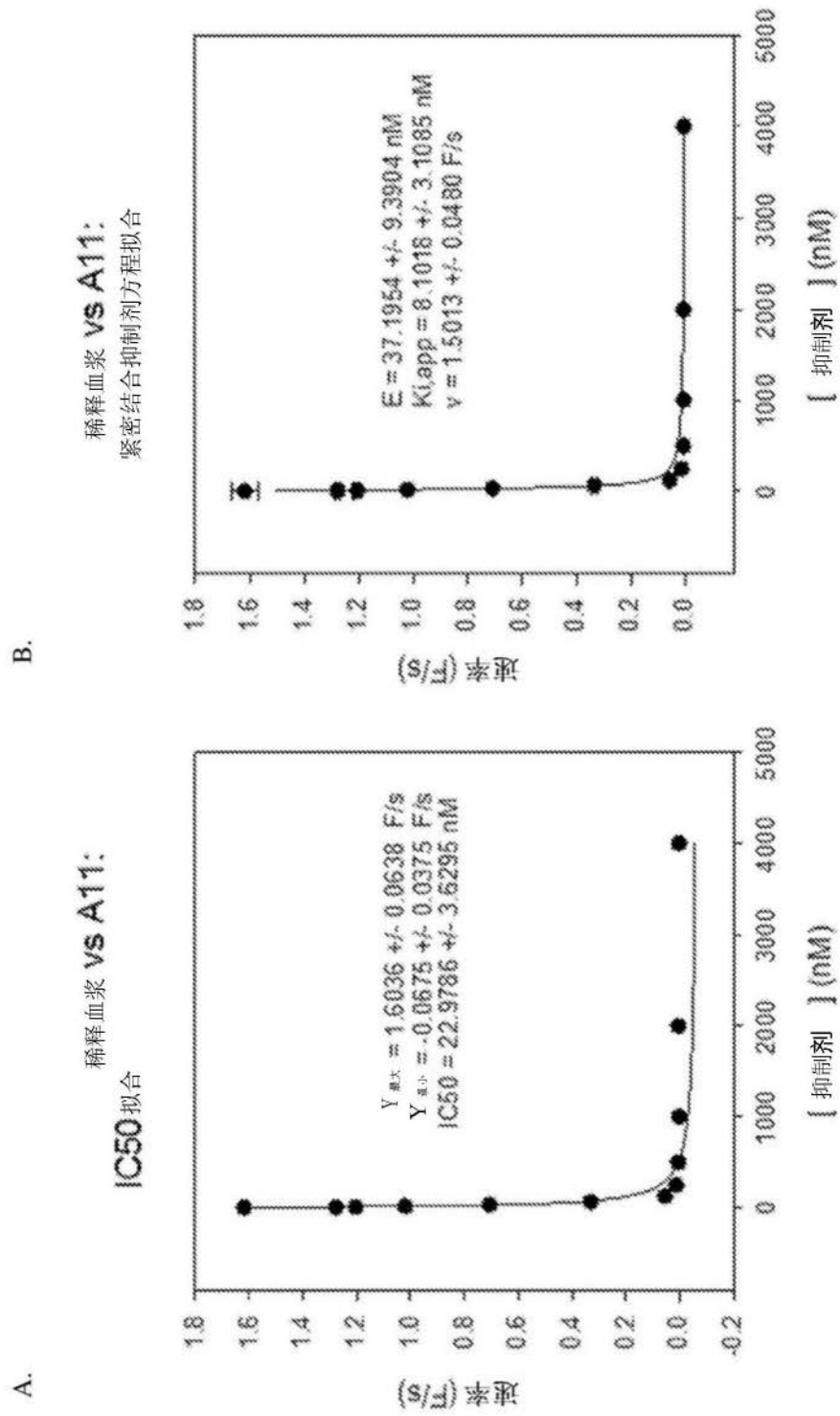


图10

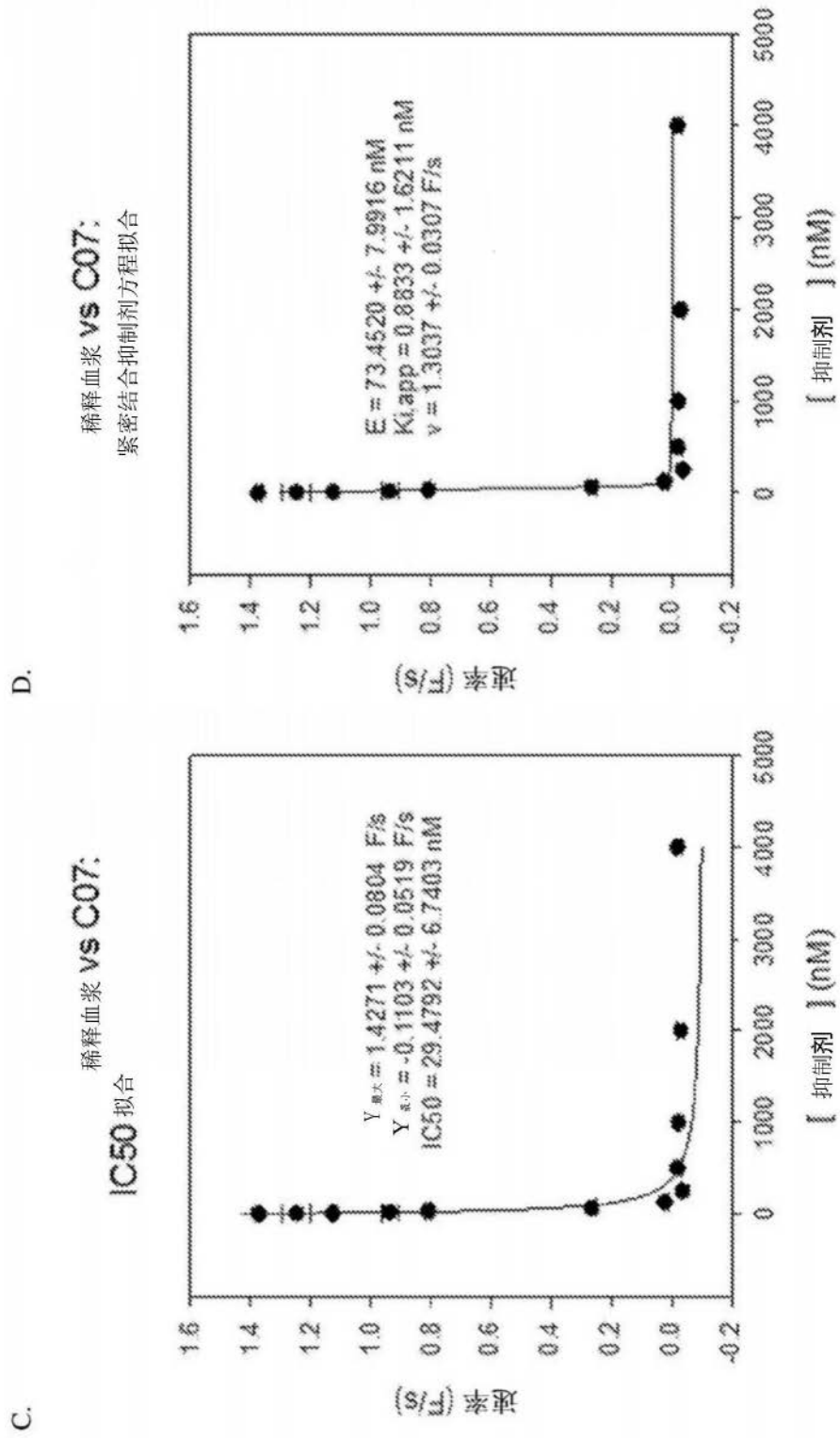


图10(续)

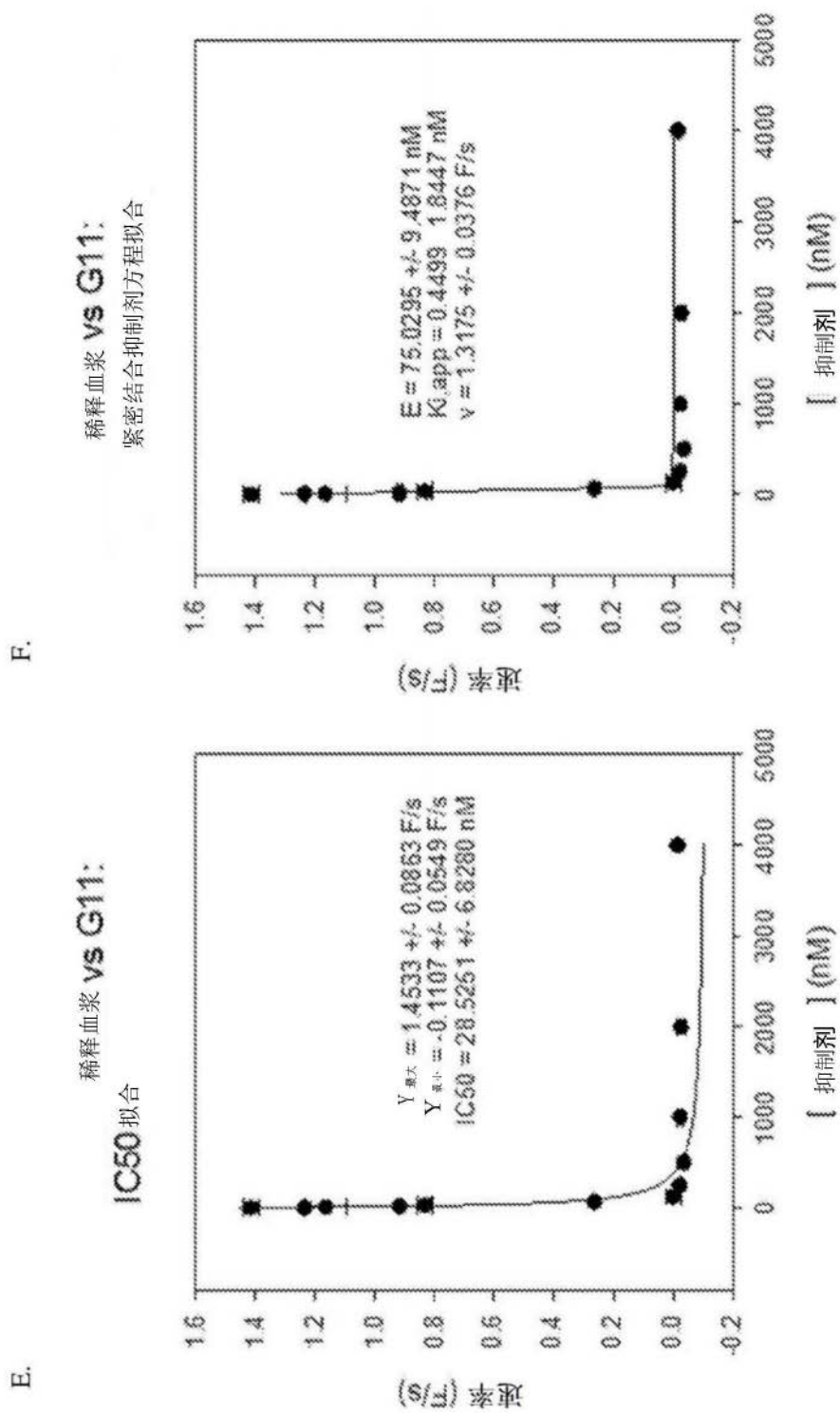


图10(续)

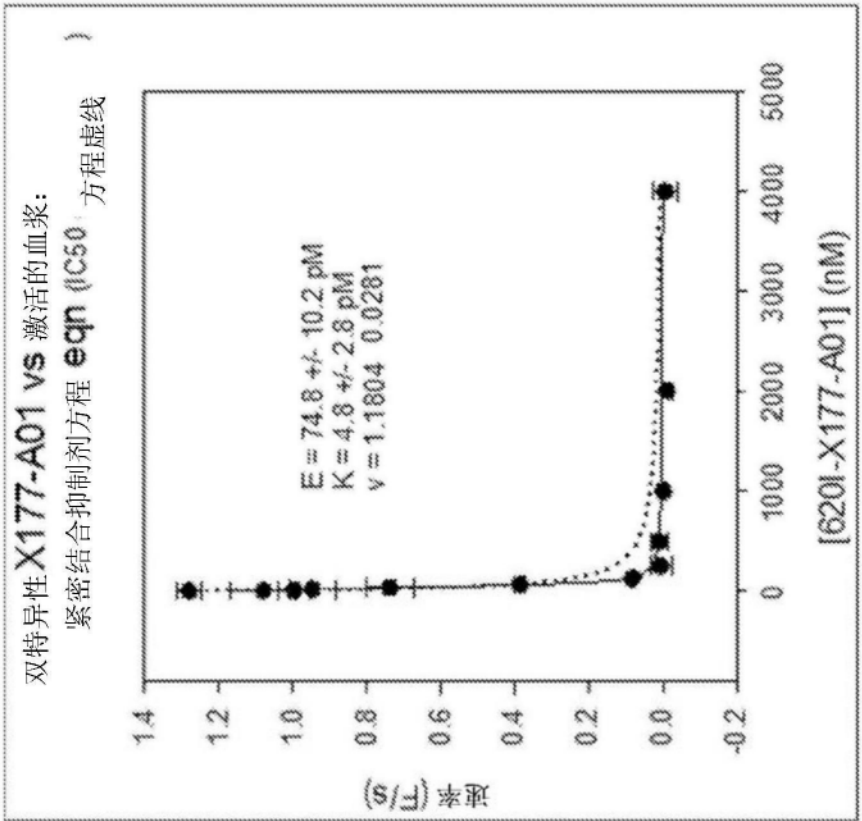


图11

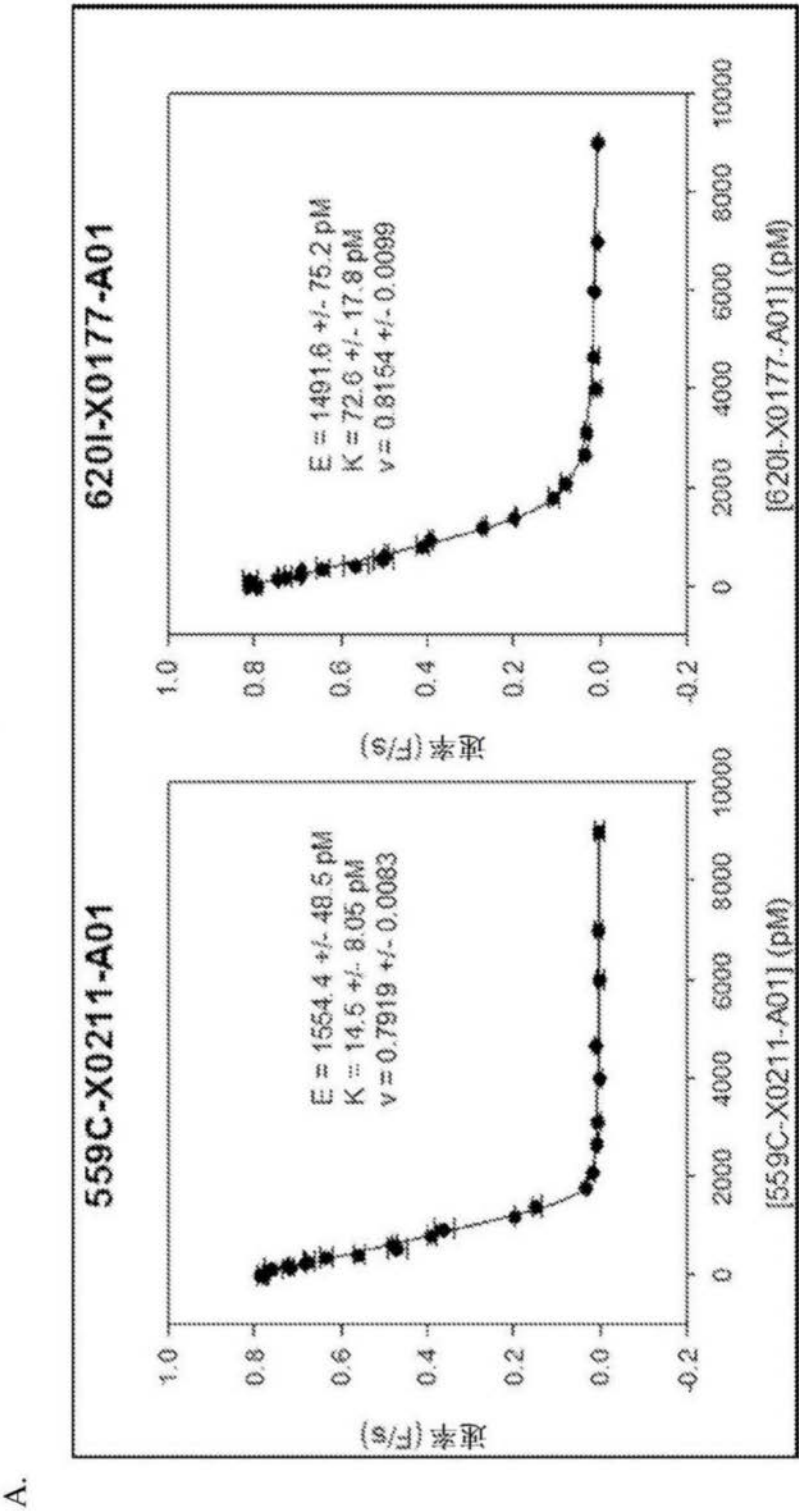
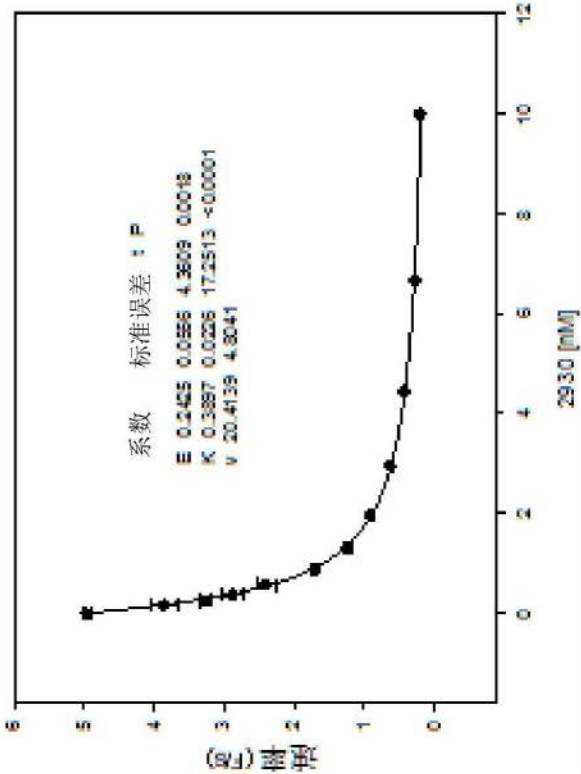


图12

620I-X0177-A01

双特异性 A01 vs hpKal
 $\sigma = ((E-I-K) + s \cdot q \cdot t((E-I-K)^2 + 4 \cdot E \cdot K)) / 2$
 $f = v \cdot C$



559C-X0211-A01

2930 vs hpKal
 $\sigma = ((E-I-K) + s \cdot q \cdot t((E-I-K)^2 + 4 \cdot E \cdot K)) / 2$
 $f = v \cdot C$

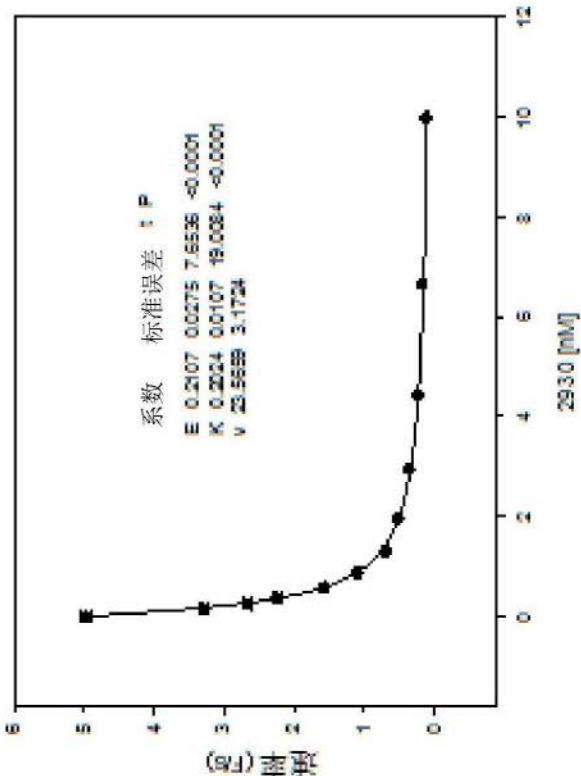


图12(续)

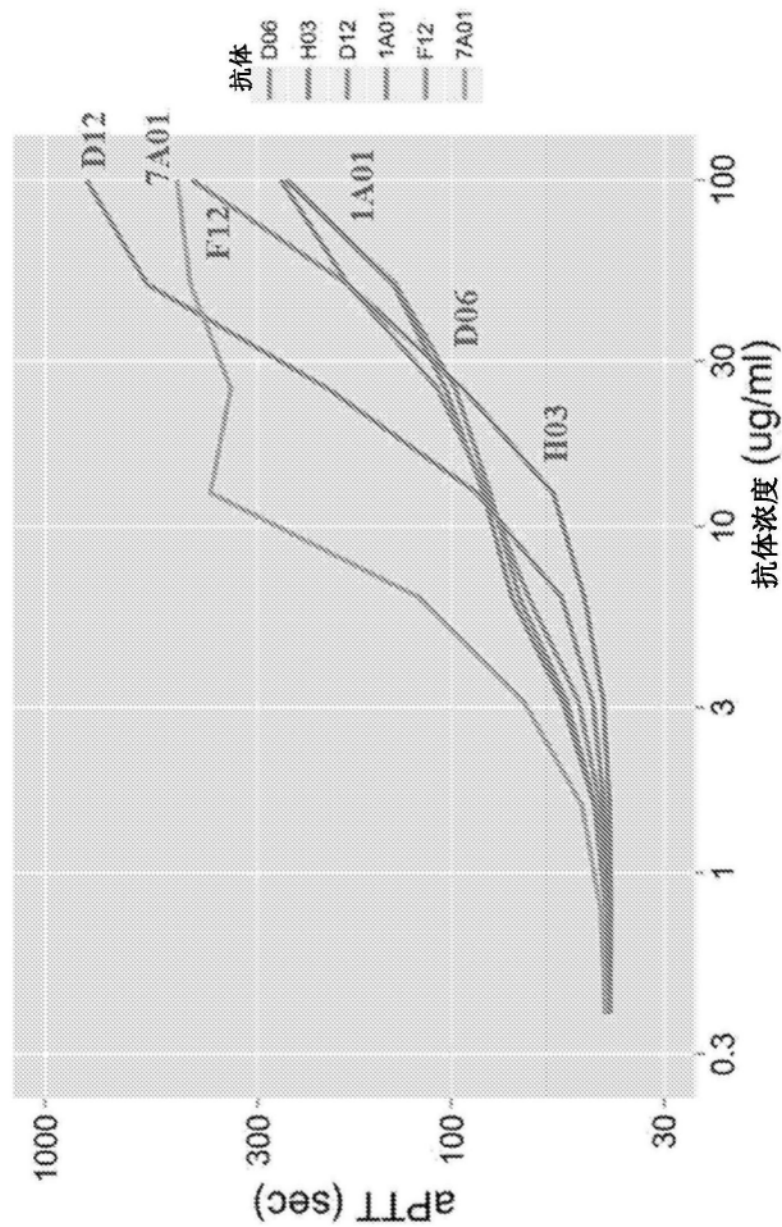


图13

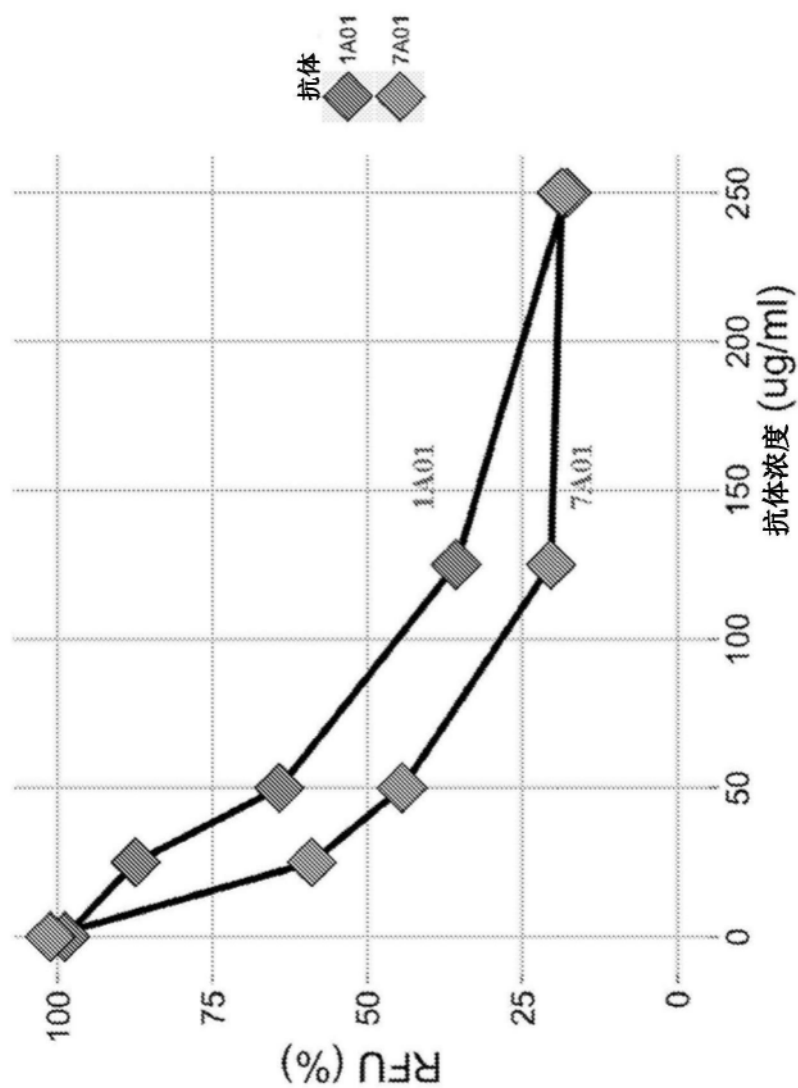
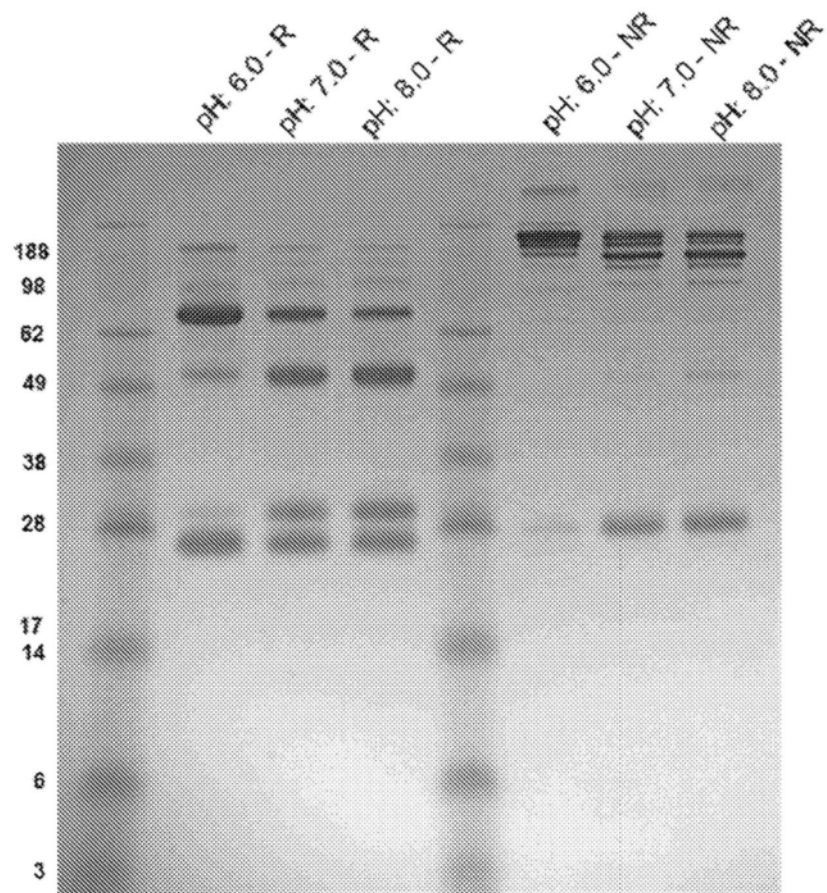


图14



SDS-PAGE

图15

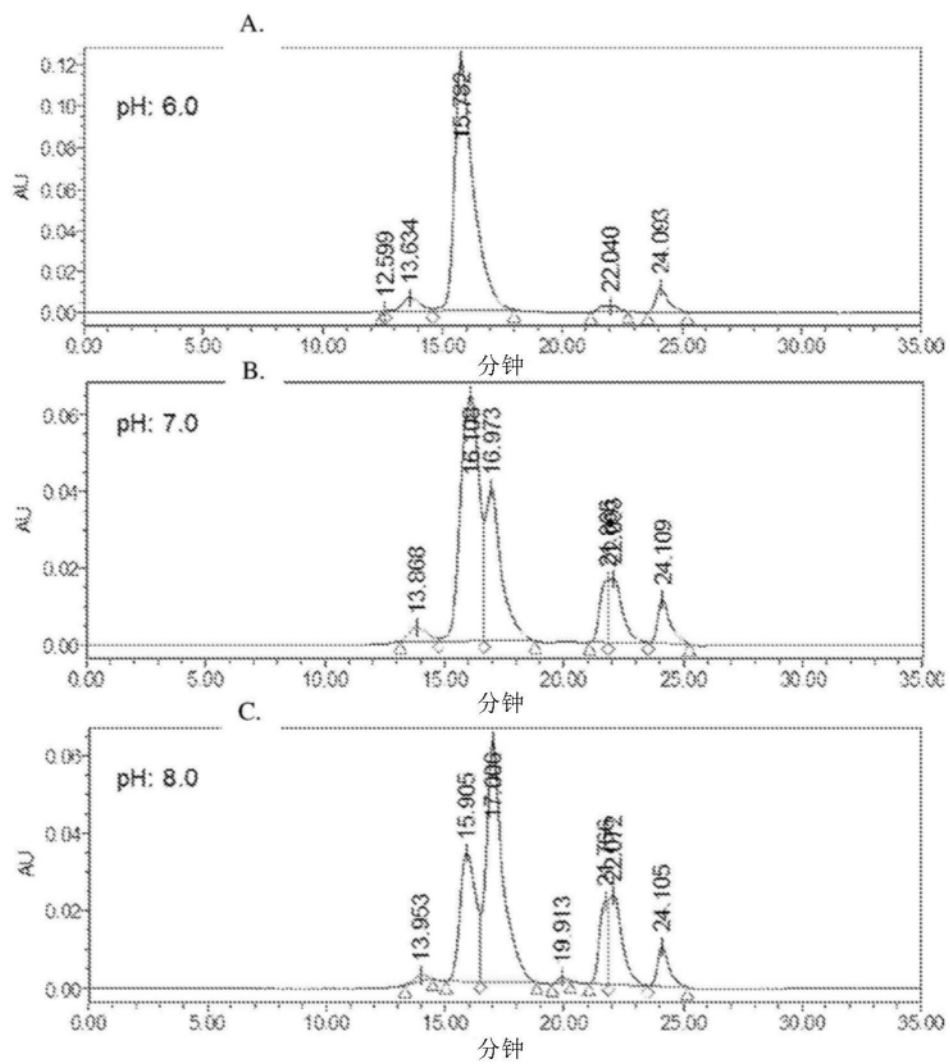


图16

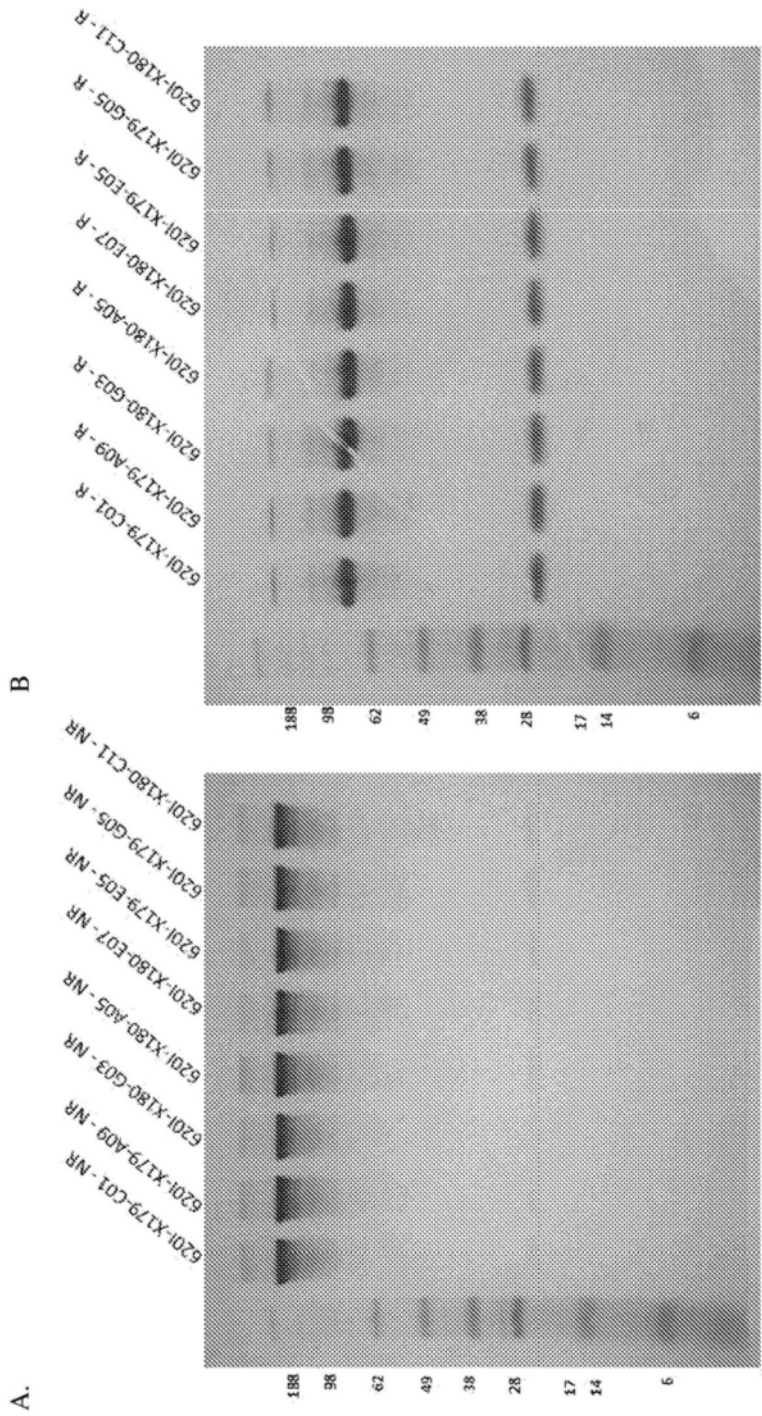


图17

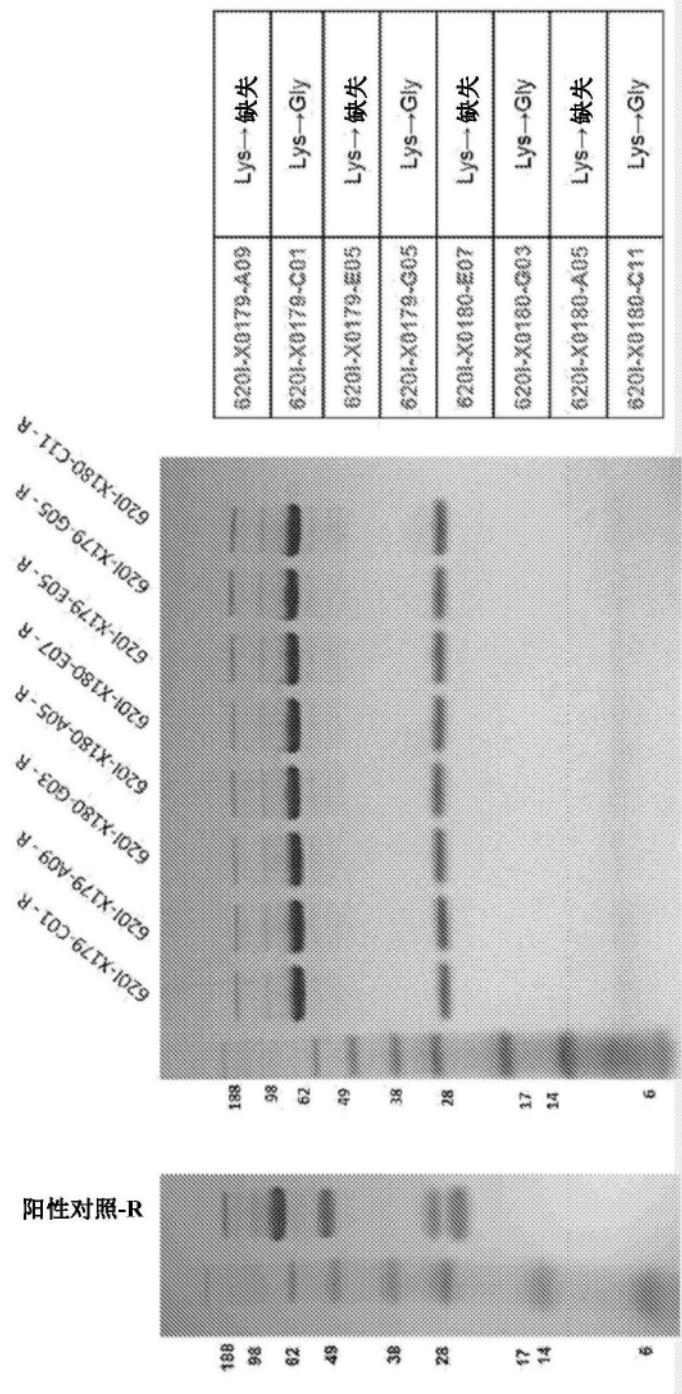


图18

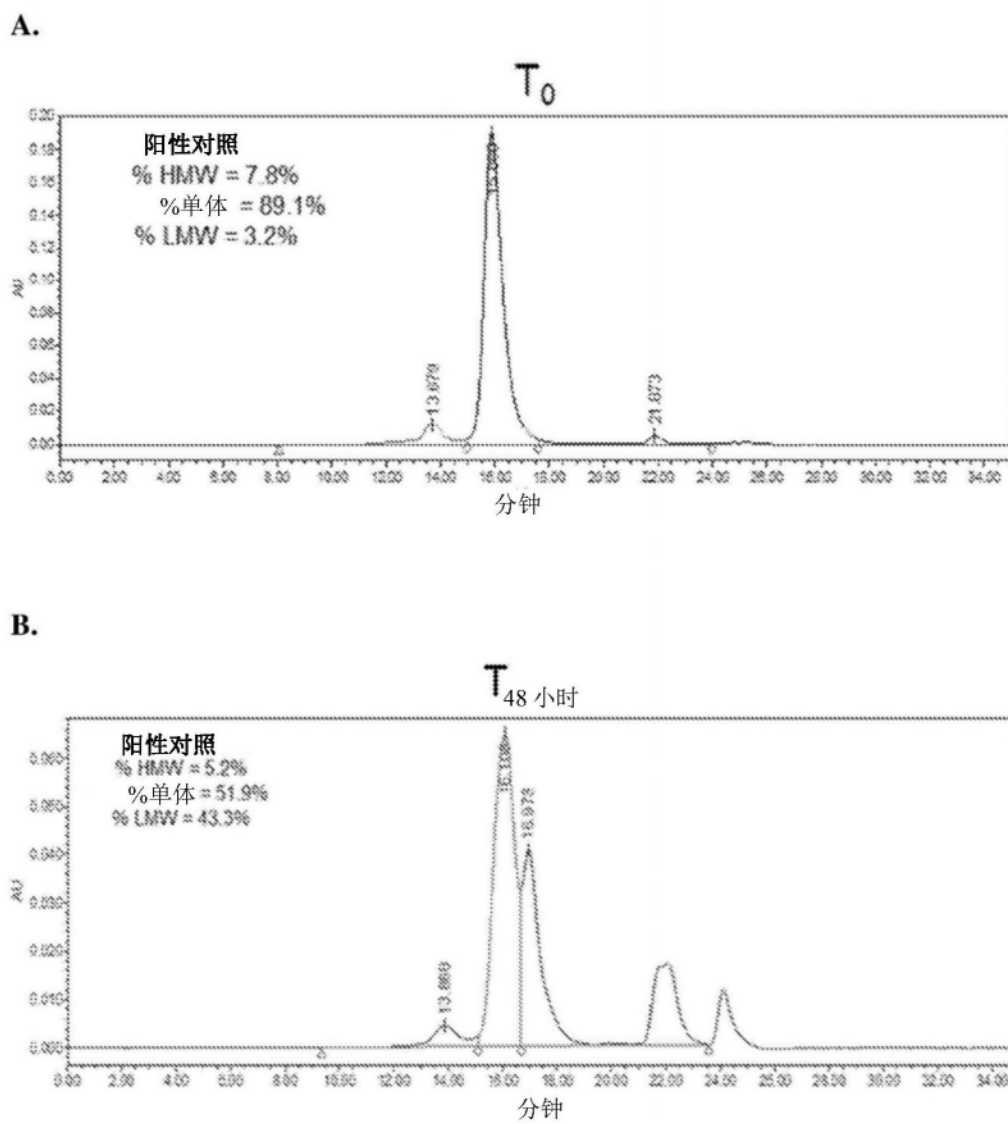


图19

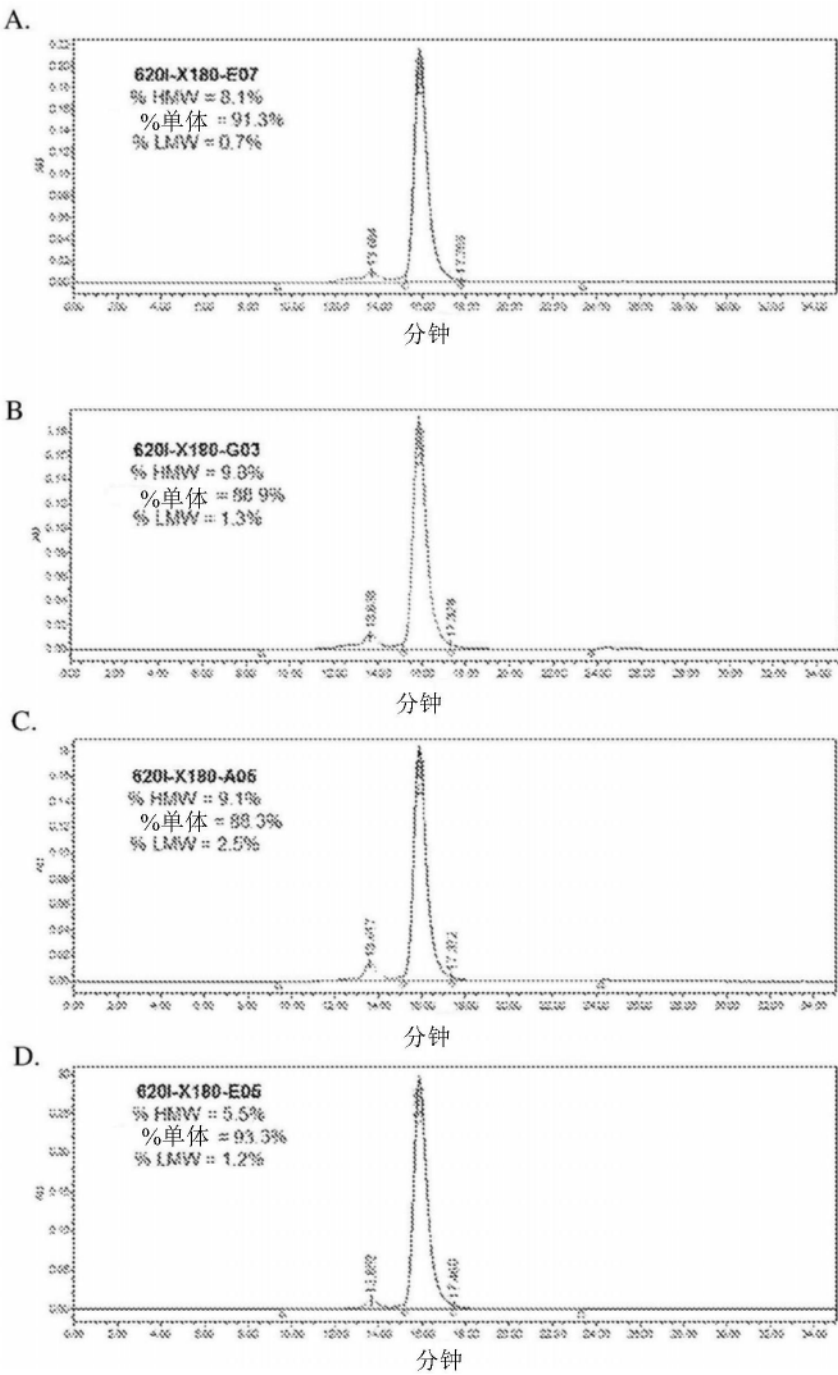


图20

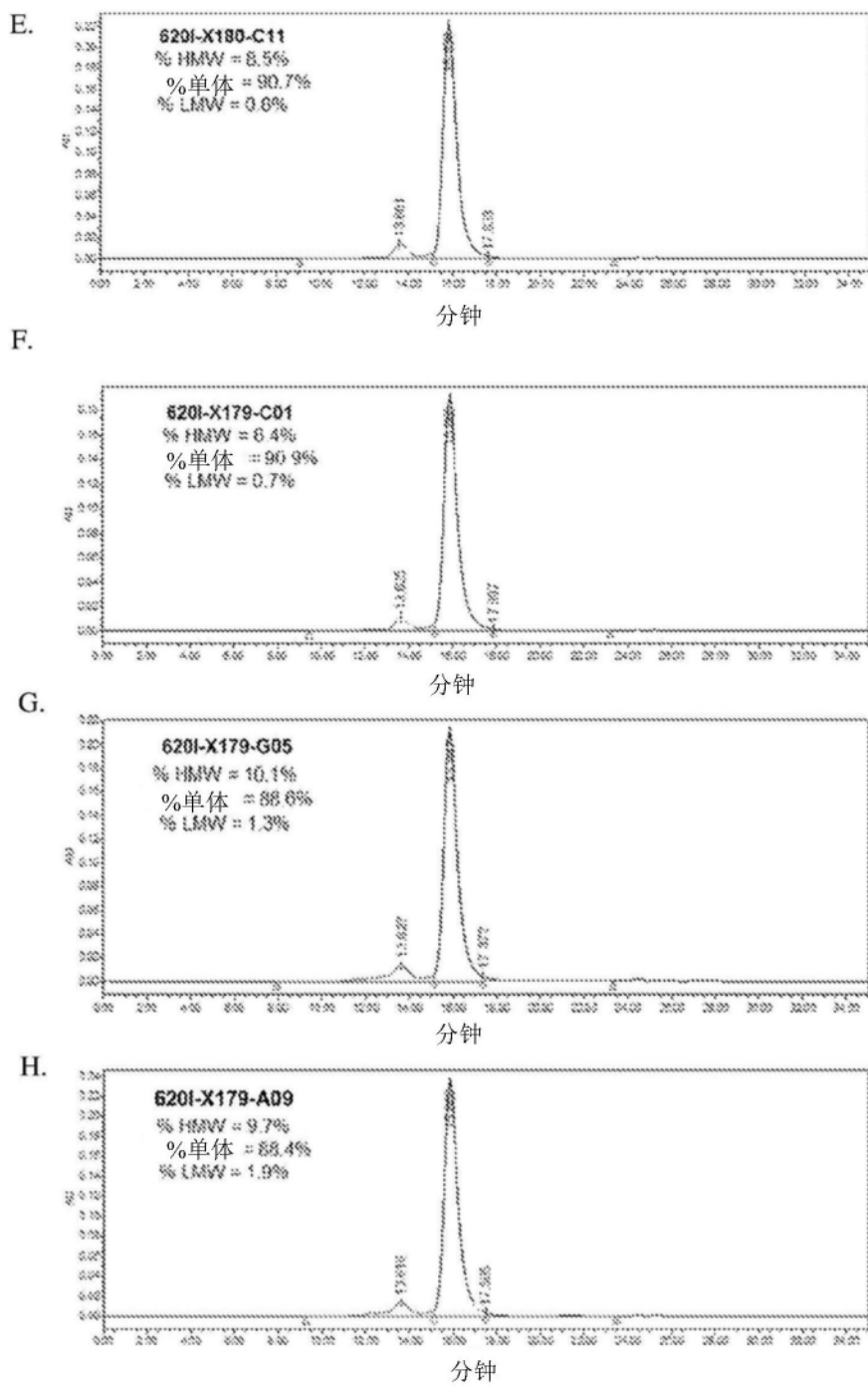


图20(续)

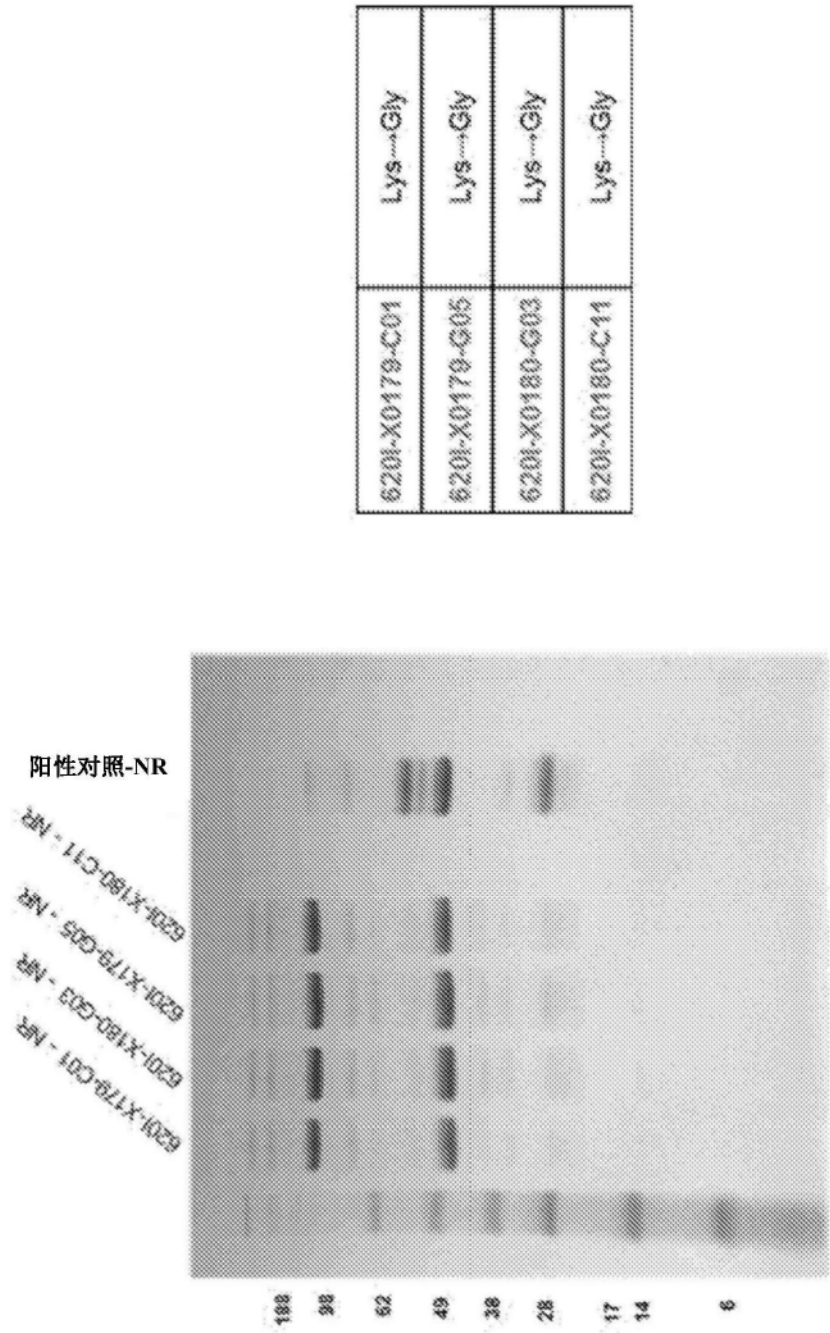


图21

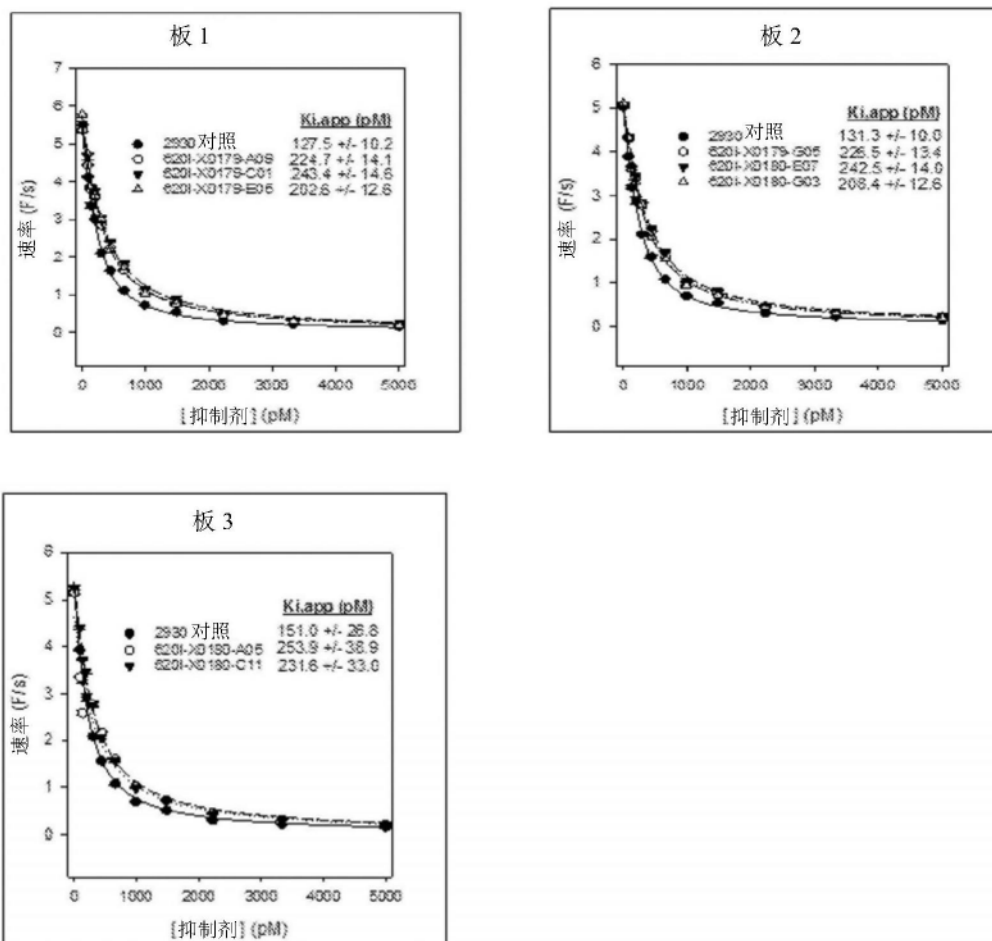


图22

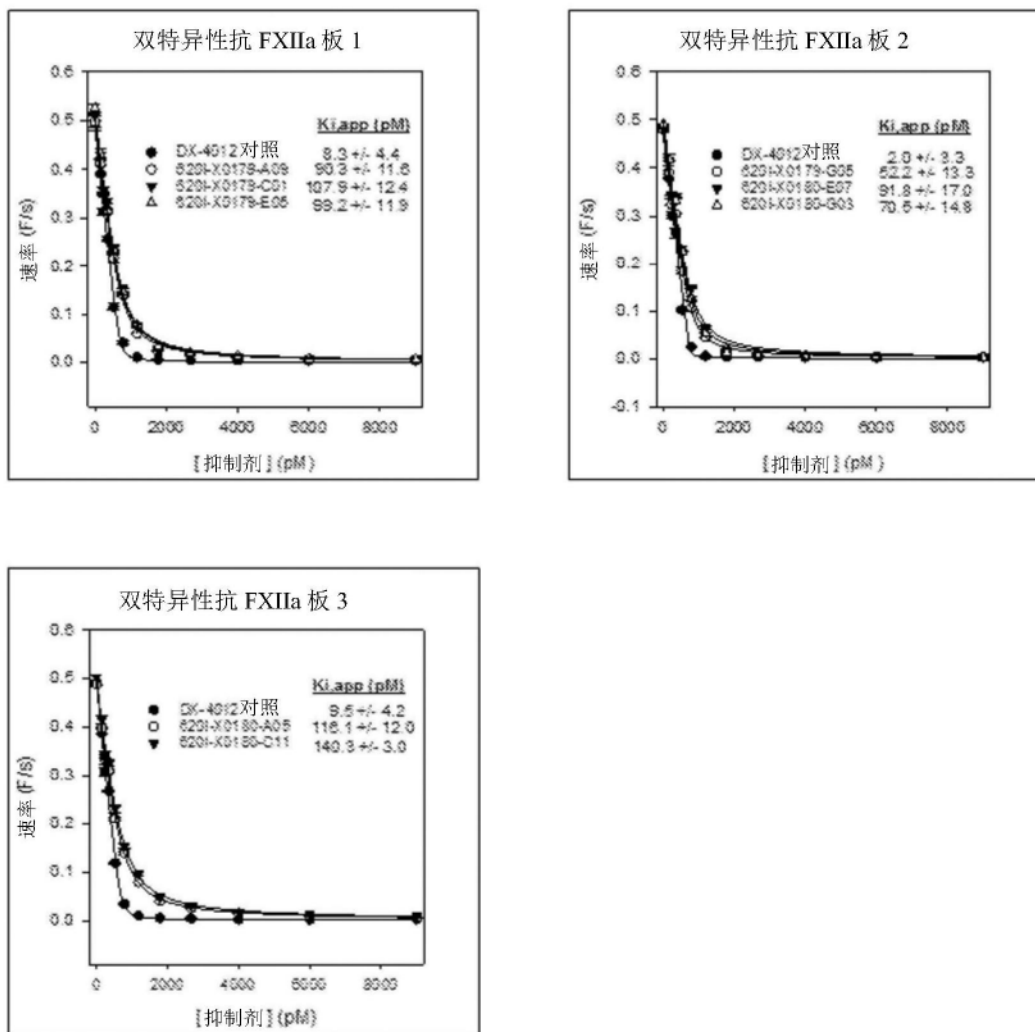


图23

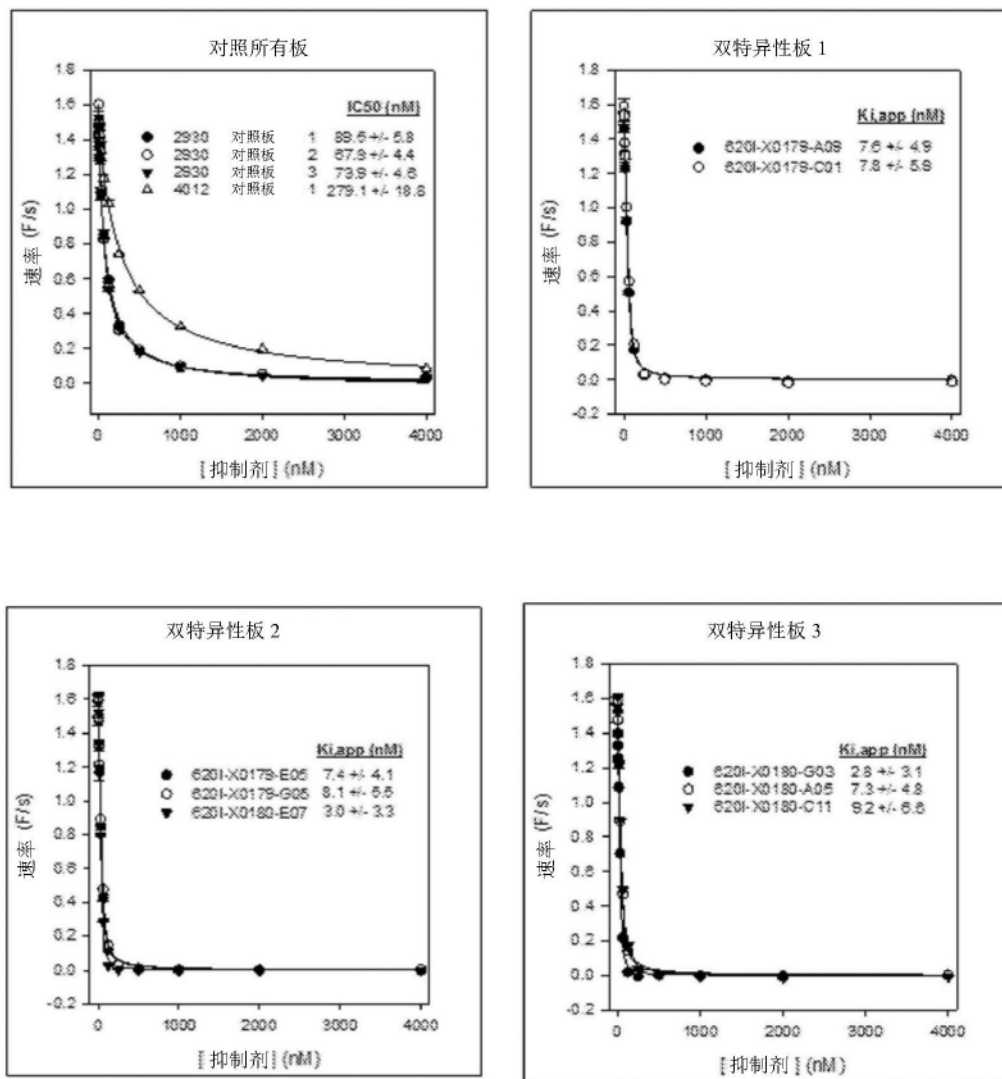


图24