

FIGURE 1

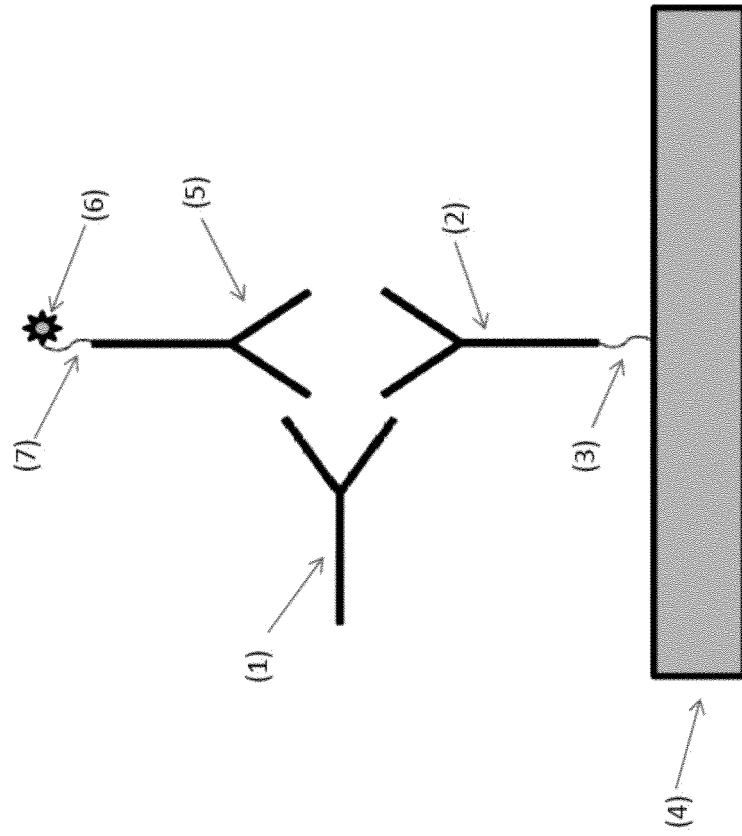


FIGURE 2A

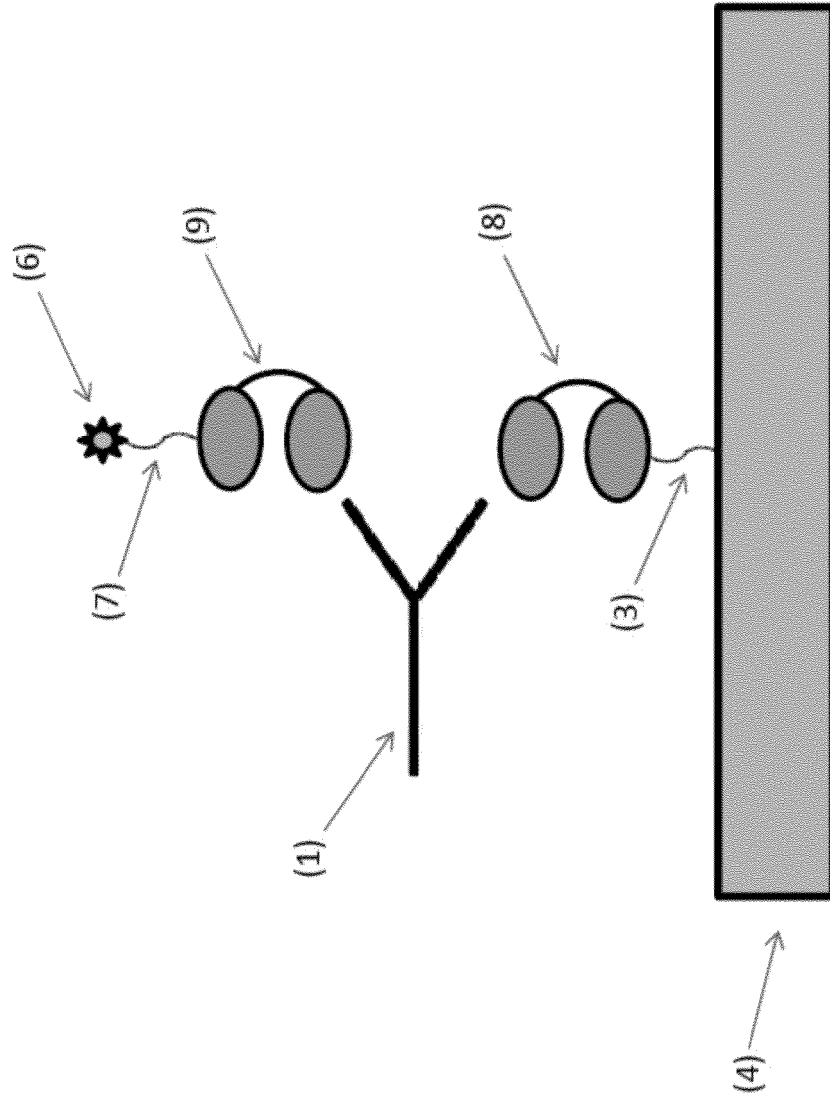


FIGURE 2B

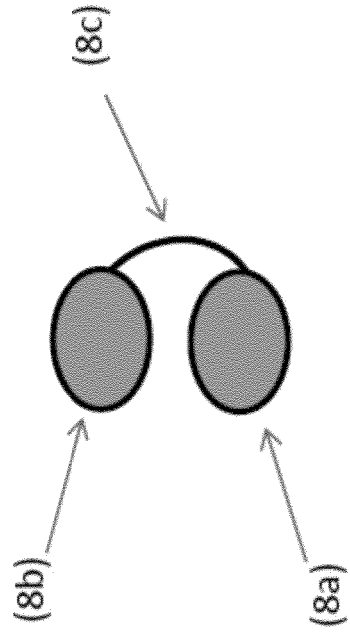


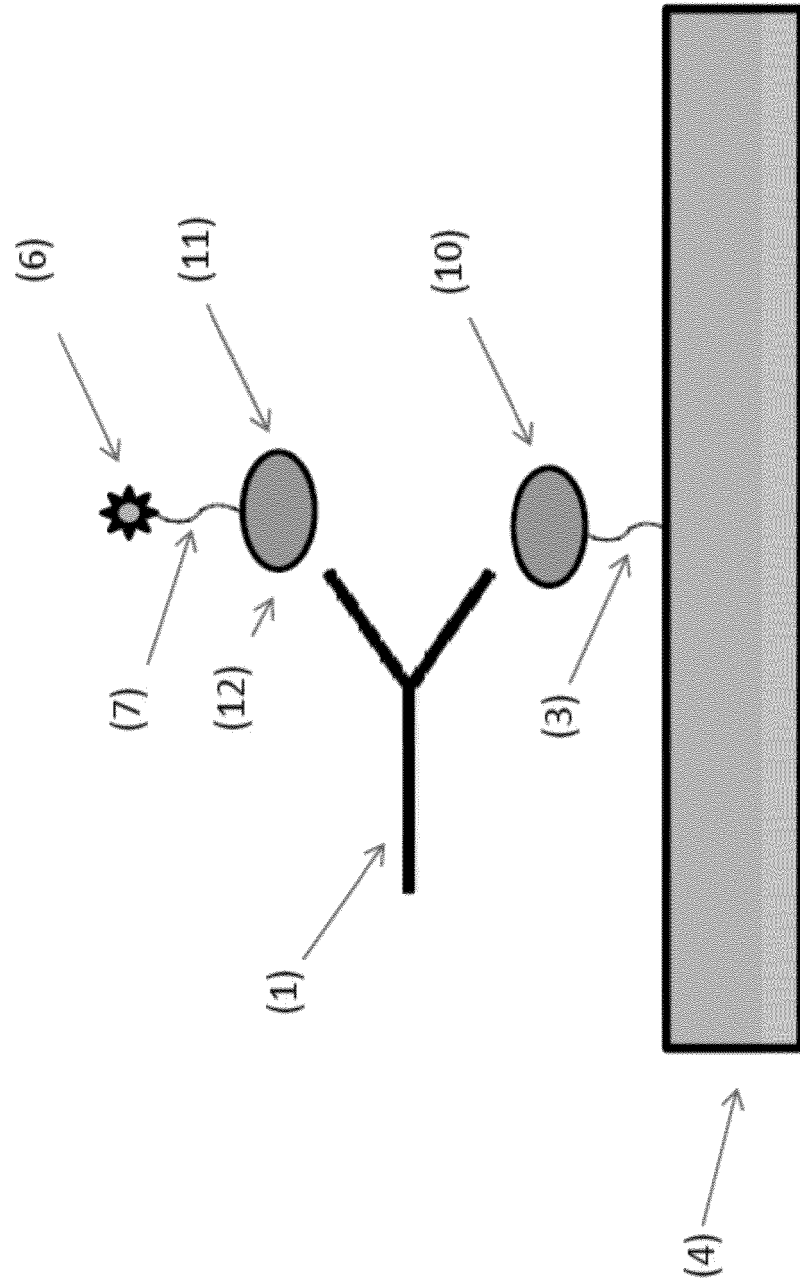
FIGURE 3

FIGURE 4

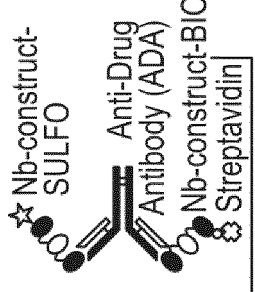
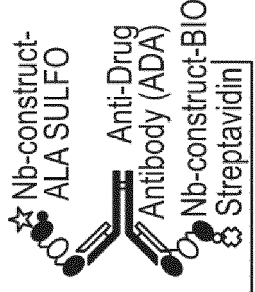
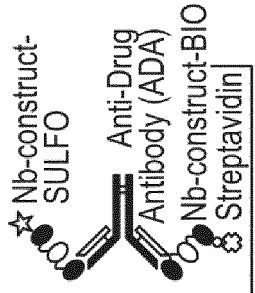
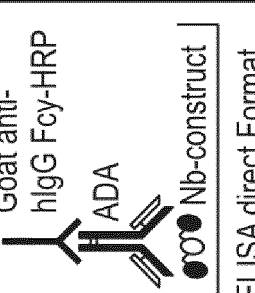
	Homogeneous ECL-based bridging	Homogeneous modified ECL-based bridging	Sequential ECL-based bridging	Sequential ELISA-based bridging	Direct ELISA format
Assay set-up	 <p>MSD Bridging Format</p>	 <p>MSD Bridging Format</p>	 <p>MSD Bridging Format</p>	 <p>ELISA Bridging Format</p>	 <p>ELISA direct Format</p>
Capture Antigen	Biotinylated Nanobody construct	Biotinylated Nanobody construct	Biotinylated Nanobody construct	Nanobody construct	Nanobody construct
Detection reagent	Sulfo-tagged Nanobody construct	Sulfo-tagged Nanobody construct-Ala (one additional Alanine at the C-terminal end of the authentic Nanobody construct)	Sulfo-tagged Nanobody construct	Biotinylated Nanobody construct & HRP conjugated Streptavidin and TMB	HRP conjugated goat anti-hlgG Fcy specific and Western Lightning ECL pro
Detection instrument	MSD Sector Imager 2400	MSD Sector Imager 2400	MSD Sector Imager 2400	Microtitre plate reader	Perkin Elmer
Positive control	anti-Nanobody construct rabbit serum (P01 rabbit anti-sera supplied by Pfizer Inc-Wyeth Research)	anti-Nanobody construct rabbit serum (P01 rabbit anti-sera supplied by Pfizer Inc-Wyeth Research)	anti-Nanobody construct rabbit serum (P01 rabbit anti-sera supplied by Pfizer Inc-Wyeth Research)	anti-Nanobody construct rabbit serum (P01 rabbit anti-sera supplied by Pfizer Inc-Wyeth Research)	anti-Nanobody construct rabbit serum (P01 rabbit anti-sera supplied by Pfizer Inc-Wyeth Research)
MRD	50 (25 + ½ in master mix)	50 (25 + ½ in master mix)	25	25	100

FIGURE 4 (CONTINUED)

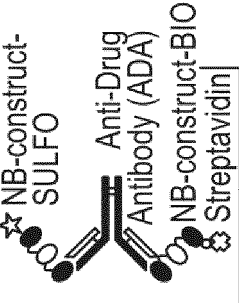
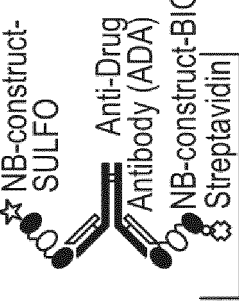
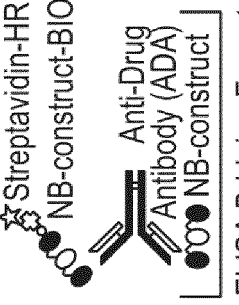
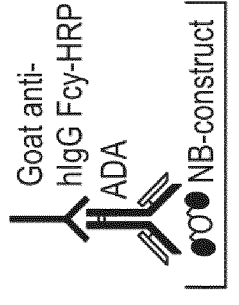
	Homogeneous ECL-based bridging	Homogeneous modified ECL-based bridging	Sequential ECL-based bridging	Sequential ELISA-based bridging	Direct ELISA format
Assay set-up	 <p>MSD Bridging Format</p>	 <p>MSD Bridging Format</p>	 <p>MSD Bridging Format</p>	 <p>ELISA Bridging Format</p>	 <p>ELISA direct Format</p>
Sensitivity	1/182,250 - 1/60,750 (=70 - 200 ng/mL, estimated based on 10mg/mL total Ig and 5% Nanobody construct specific)	1/182,250 - 1/60,750 (=70 - 200 ng/mL, estimated based on 10mg/mL total Ig and 5% Nanobody construct specific)	1/2,250 (=5.5 µg/mL, estimated based on 10mg/mL total Ig and 5% Nanobody construct specific)	1/20,250 (=600 ng/mL; estimated based on 10mg/mL total Ig and 5% Nanobody construct specific)	1/20,250 (=600 ng/mL; estimated based on 10mg/mL total Ig and 5% Nanobody construct specific). Note that estimate is based on rabbit serum and HRP-conjugated anti-rabbit-IgG detector.
Cut-point setting remarks	Cut-point setting is arbitrary. Commonly used statistical methods are not applicable	possible	possible	Cut-point setting is arbitrary. Commonly used statistical methods are not applicable	Cut-point setting is arbitrary. Commonly used statistical methods are not applicable
Detection of positive control in presence of interference	Not possible	Only samples with high interference might defy detection of low ADA levels (ng range).	Samples with high interference might defy detection of high ADA levels (µg range)	Not possible	Technically not feasible (due to different detection reagents for rabbit-positive control and interference)
Conclusion	impacted by interference	Allows for detection of ADA in the presence of interference	Impacted by interference	Impacted by interference	Impacted by interference

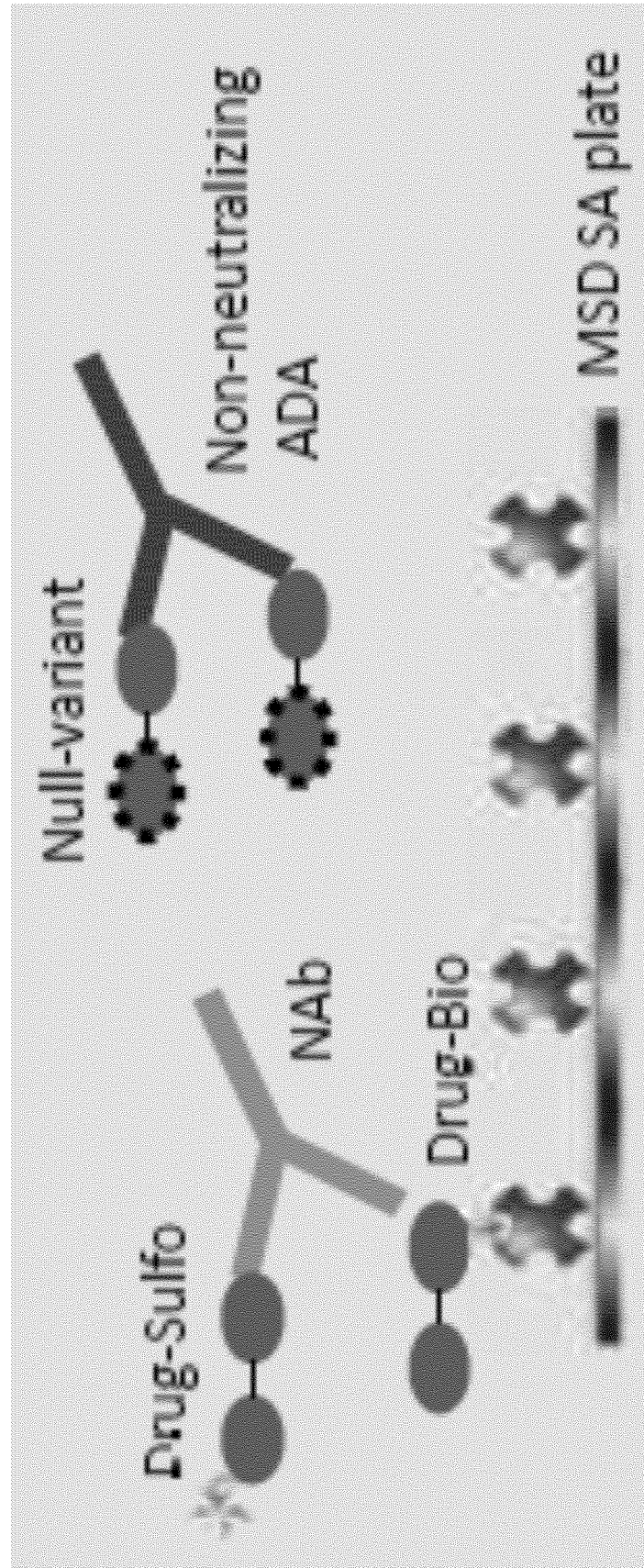
FIGURE 5

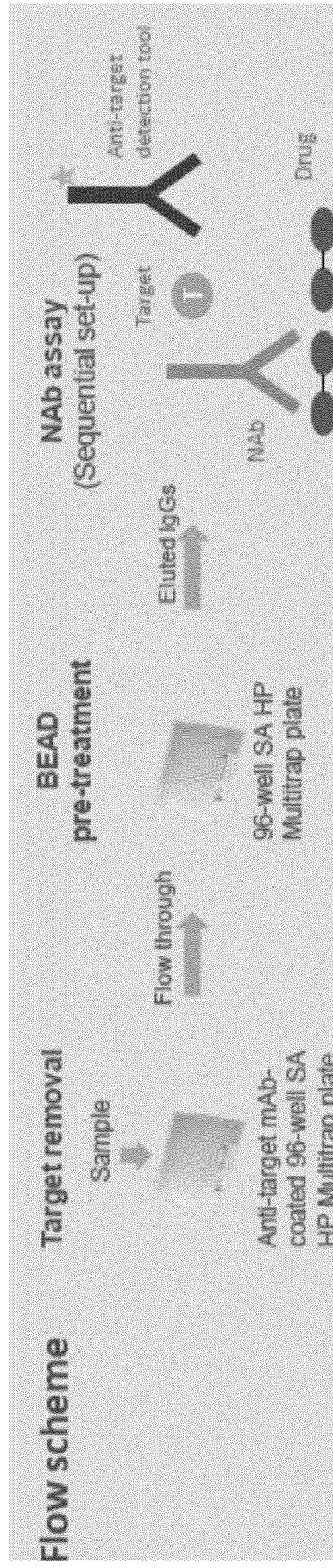
FIGURE 6

FIGURE 7

Target	ISV-based drug	Corresponding null variant
vWF	EVQLVESGGGLVQPGGSLRLSCAASGRTFSYNPM GWRQAPGKGRELVAISR TGGSTYYPDSVEGRF TISRDNAKRMVYLQMNSLRAEDTAVYYCAAAG VRAEDGRVRTLPSEYTFWGQGTQVTVSSAAAEV QLVESGGGLVQPGGSLRLSCAASGRTFSYNPMG WFRQAPGKGRELVAISR TGGSTYYPDSVEGRFT ISRDNAKRMVYLQMNSLRAEDTAVYYCAAAGV RAEDGRVRTLPSEYTFWGQGTQVTVSS (SEQ ID NO:1)	EVQLVESGGGLVQPGGSLRLSCAASGRYFRENPM GWRQAPGKGRELVAISR TGGSTYYPDSVEGRF TISRDNAKRMVYLQMNSLRAEDTAVYYCAAAG VRATDGRVRTLPSEYTFWGQGTQVTVSSAAAEV QLVESGGGLVQPGGSLRLSCAASGRYFRENPMG WFRQAPGKGRELVAISR TGGSTYYPDSVEGRFT SRDNAKRMVYLQMNSLRAEDTAVYYCAAAGVR ATDGRVRTLPSEYTFWGQGTQVTVSS (SEQ ID NO:2)
IL6-R	EVQLVESGGGLVQPGGSLRLSCAASGVFKINVM AWYRQAPGKGRELVAGISGGSTSYADSVKGRFT ISRDNAKNTLYLQMNSLRPEDTAVYYCAFITTES DYDLGRRYWGQGTQVTVSSGGGGGGGSEVQLV ESGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQ APGKGLEWVSSISGSGSDTLYADSVKGRFTISRDN AKTTLYLQMNSLRPEDTAVYYCTIGGSLRSSQG TLVTVSS (SEQ ID NO:3)	EVQLVESGGGLVQPGGSLRLSCAASGVFGSNVM AWYRQAPGKGRELVAGISKGSTSYADSVKGRFT ISRDNAKNTLYLQMNSLRPEDTAVYYCAFITTIRD YDLGREYWGQGTQVTVSSGGGGGGGSEVQLVE SGGGLVQPGNSLRLSCAASGFTSSFGMSWVRQA PGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNA KTTLYLQMNSLRPEDTAVYYCTIGGSLRSSQGT LVTVSS (SEQ ID NO:4)

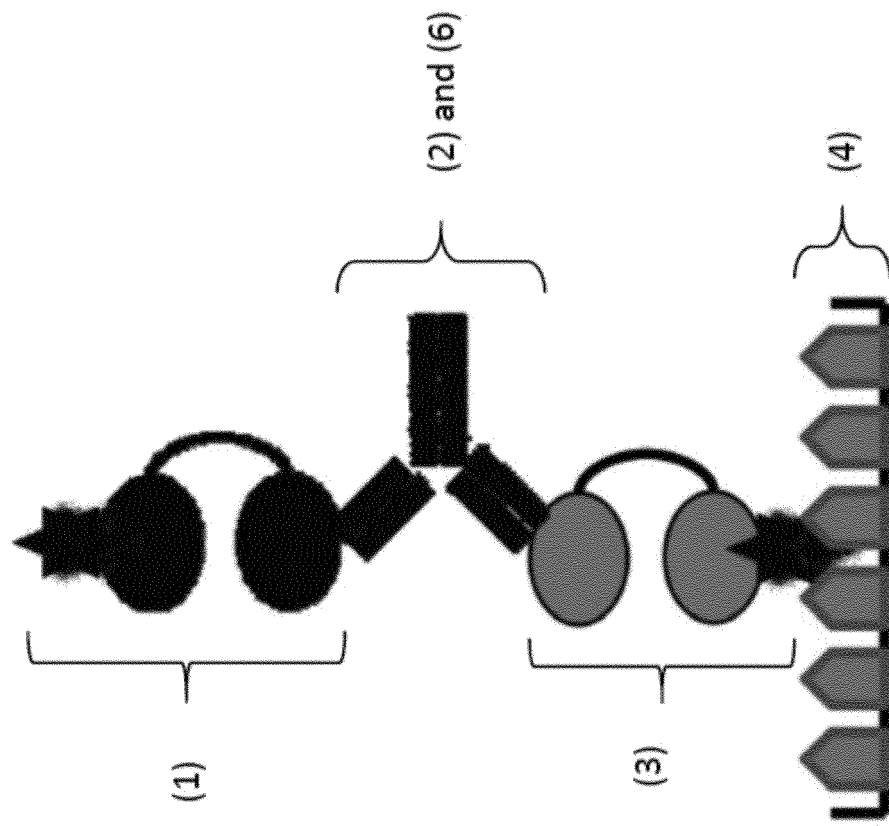
FIGURE 8A

FIGURE 8B

