Structural Modeling and *In Silico* Screening of Potential Small Molecule Allosteric Agonists of Glucagon-Like Peptide 1 Receptor

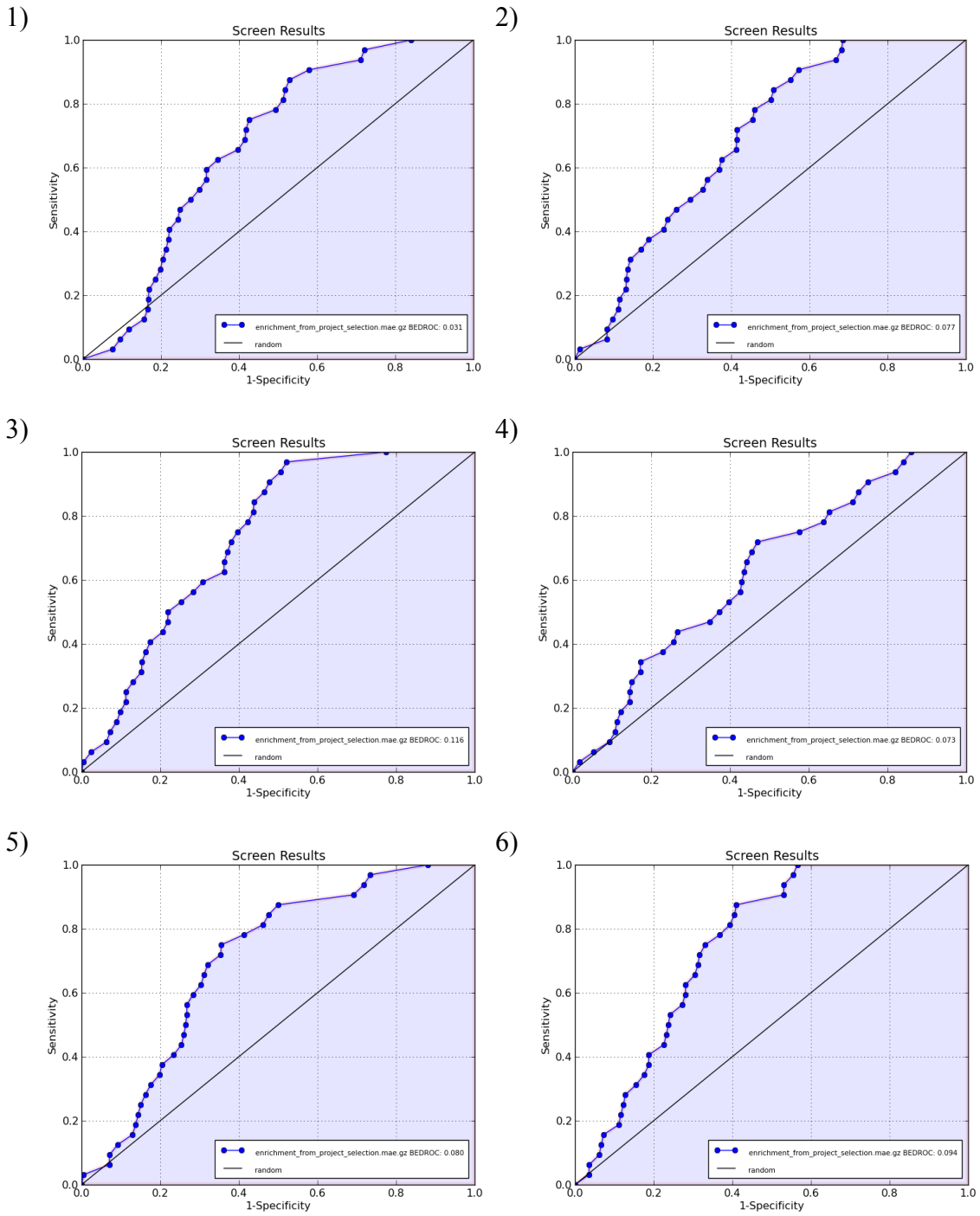
Tejashree Redij[#,£], Rajan Chaudhari[¶,£,1], Zhiyu Li[$], Xianxin Hua[§], and Zhijun Li[#,¶,*]

[#]Department of Biological Sciences, University of the Sciences in Philadelphia, Philadelphia, PA 19104, USA.
[¶]Department of Chemistry & Biochemistry, University of the Sciences in Philadelphia, Philadelphia, PA 19104, USA.
[$]Department of Pharmaceutical Sciences, University of the Sciences in Philadelphia, Philadelphia, PA 19104, USA.
[§]Department of Cancer Biology, Diabetes Research Center (DRC), University of Pennsylvania, Philadelphia, PA 19104, USA.

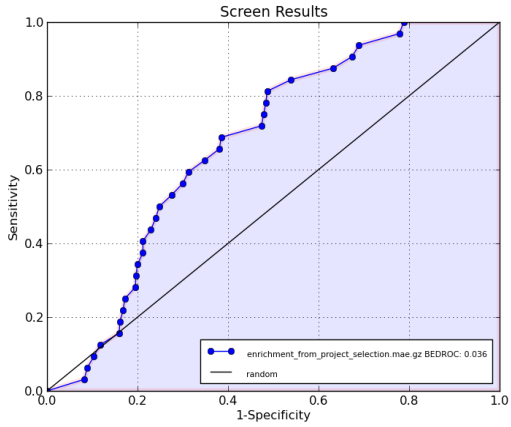
[£]Both authors contributed equally to this work.
*To whom correspondence should be addressed.
Address: Department of Chemistry & Biochemistry, Box 48, University of the Sciences in Philadelphia, Philadelphia, PA 19104, USA
Phone: 215-596-7539
Fax: 215-895-1112
E-mail: z.li@usciences.edu

[1]Current address: Experimental Therapeutics Department, University of Texas MD Anderson Cancer Center, 1881 East Rd, Unit 1950, 3SCR4.3426, Houston, TX 77054

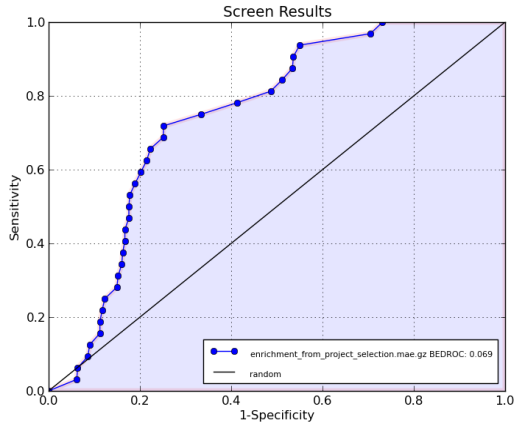
Supplementary Figure S1. The ROC plots of ten distinct conformations of GLP-1R from conformation sampling.



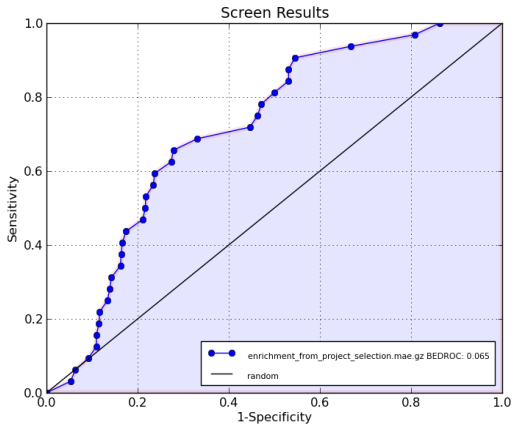
7)


Screen Results
Sensitivity
1-Specificity
enrichment_from_project_selection.mae.gz BEDROC: 0.036
random

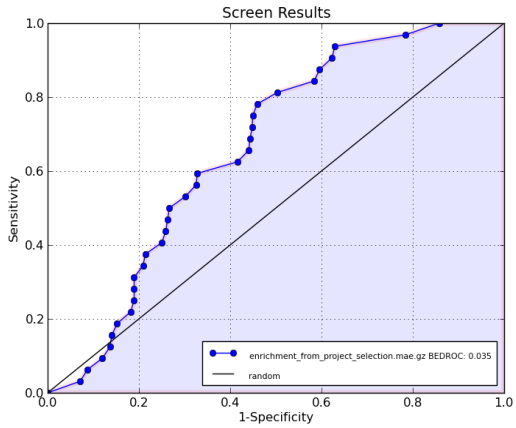
8)


Screen Results
Sensitivity
1-Specificity
enrichment_from_project_selection.mae.gz BEDROC: 0.069
random

9)


Screen Results
Sensitivity
1-Specificity
enrichment_from_project_selection.mae.gz BEDROC: 0.065
random

10)


Screen Results
Sensitivity
1-Specificity
enrichment_from_project_selection.mae.gz BEDROC: 0.035
random

Supplementary Figure S2. Sequence alignment of residues in the predicted binding site between human GLP-1R and its most similar Class B GPCRs. Residues that are different between GLP-1R and VIPR1 were highlighted.

	2.60x60	3.43x43	3.44x44	3.47x47	5.40x40	5.47x47	5.50x50	5.51x51	6.45x45	6.46x46	6.52x52	6.53x53
Human GLP-1R	R	N	Y	L	R	I	N	F	L	I	H	E
Human GCGR	K	N	Y	L	R	I	N	F	L	I	H	E
Human GIPR	R	N	Y	L	R	I	N	F	L	V	H	E
Human GLP-2R	R	N	Y	L	R	V	N	F	L	I	H	E
Human Secretin Receptor	R	N	Y	L	R	I	N	F	L	I	H	Y
Human PAC1R	R	N	Y	L	K	I	N	F	L	I	H	Y
Human VIPR1	R	N	F	L	K	I	N	F	L	I	H	Y
Human VIPR2	R	N	F	L	R	I	N	F	L	I	H	Y

Supplementary Figure S3. VIPR peptide agonist mediated VIPR signaling and VIPR peptide antagonist mediated VIPR inhibition in HEK293 cells expressing the cAMP response element-luciferase reporter. HEK293-CREB luciferase cells were treated with different concentrations of VIPR peptide agonist in the absence or presence of VIPR peptide antagonist. VIPR activation was measured and plotted as the amount of luminescence produced, which was normalized by protein concentration. The readings for VIPR peptide agonist alone are plotted as (VIPR peptide alone). The combination of VIPR peptide agonist and antagonist is represented as (VIPR agonist + VIPR antagonist 1.12μM) and (VIPR agonist + VIPR antagonist 5.6 μM). The data indicated that VIPR antagonist at 5.6 μM is effective to deactivate VIPR signaling by VIPR peptide agonist. Data showed that in the presence of VIPR in HEK293 and VIPR activity could be effectively inhibited by VIPR antagonist. This assay can be used to evaluate the non-specific contribution of VIPR in response to M_4. Data is representative of three independent experiments with at least three technical replicates for each treatment conditions and error bars for each concentration were plotted as SEM (n=3). Statistical analysis was done using 2-way ANOVA (****p<0.0001). The statistical comparison was done with respect to the corresponding normalized luminescence reading of VIPR activation by VIPR peptide agonist alone.