



Universiteit
Leiden
The Netherlands

Application of fragment-based drug discovery to membrane proteins Früh, V.

Citation

Früh, V. (2009, October 7). *Application of fragment-based drug discovery to membrane proteins*. Retrieved from <https://hdl.handle.net/1887/14047>

Version: Not Applicable (or Unknown)

License: [Leiden University Non-exclusive license](#)

Downloaded from: <https://hdl.handle.net/1887/14047>

Note: To cite this publication please use the final published version (if applicable).

Application of fragment-based drug discovery to membrane proteins

Application of fragment-based drug discovery to membrane proteins

Proefschrift

ter verkrijging van

de graad van Doctor aan de Universiteit Leiden,

op gezag van Rector Magnificus Prof. Mr. P.F. van der Heijden,

volgens besluit van het College voor Promoties

te verdedigen op woensdag 7 Oktober 2009

klokke 16.15 uur

door

Virginie Früh

geboren te Lausanne in 1978

PROMOTIECOMMISSIE

Promotor: Prof. Dr. A. P. IJzerman

Co-Promotor: Dr. G. Siegal

Overige leden: Prof. Dr. J-L. Popot

Prof. Dr. C.A.A. van Boeckel

Prof. Dr. G. Canters

Prof. Dr. J. Brouwer

Prof. Dr. M. Danhof

This research described in this thesis was jointly funded by and performed at the Division of Medicinal Chemistry of Leiden/Amsterdam Center for Drug Research, Leiden University (Leiden, the Netherlands) and the Leiden Institute of Chemistry, Leiden University (Leiden, the Netherlands).

This thesis was printed by Wöhrmann Print Service (Zutphen, The Netherlands).

"A chemist walks into a pharmacy and asks the pharmacist,

"Do you have any acetylsalicylic acid?"

"You mean aspirin?" asked the pharmacist.

"That's it, I can never remember that word."

Q: if both a bear in Yosemite and one in Alaska

fall into the water

which one dissolves faster?

A: The one in Alaska because it is Polar.

To my family:

Who taught me that laughter is always the best medicine!

Table of Contents

ABBREVIATIONS		7
CHAPTER 1	General introduction	11
CHAPTER 2	How to catch a membrane protein in action: A review of functional membrane protein immobilization strategies and their applications	37
CHAPTER 3	Functional immobilization of histamine H ₁ and adenosine A ₁ receptors on sepharose beads: A facile approach with broad applicability to membrane proteins	75
CHAPTER 4	Target-immobilized NMR Screening: Validation and extension to membrane proteins	99
CHAPTER 5	Application of fragment based drug discovery to identify inhibitors of the membrane enzyme DsbB	129
CHAPTER 6	Self assembly of protein – nanodisc complexes: a solubilization strategy which enables fragment based drug discovery of membrane proteins in aqueous buffers	157
CHAPTER 7	General conclusions & Perspectives	169
REFERENCES		183
SUMMARY		206
SAMENVATTING		210
RESUME		214
APPENDICES	Curriculum Vitae	219
	List of publications	220
	Acknowledgements	221

Abbreviations

5-HT ₃ R	serotonin-gated mouse ion channel 5-hydroxytryptamine type-3 receptor
8-CPT	8-cyclopentyl-1,3-dimethylxanthin
ADA	adenosine deaminase
ATP	adenosine triphosphate
AMPPNP	adenyl-5'-yl imidodiphosphate
CB	cytochalasin B
CcO	cytochrome c oxidase
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
CHS	cholesteryl hemisuccinate
cLogP	logarithm of the partition coefficient between <i>n</i> -octanol and water
CMC	critical micellar concentration
CPA	<i>N</i> ⁶ -cyclopentyladenosine
CTAC	etyltrimethylammonium chloride
CTAB	etyltrimethylammonium bromide
DDM	dodecyl- <i>n</i> -β-maltoside
DHPC	dihexanoylphosphatidylcholine
DMPC	dimyristylphosphatidylcholine
DMPE	1,2-dimyristoyl- <i>sn</i> -glycero-phosphatidylethanolamine
DMSO	dimethyl sulfoxide
DPC	dodecylphosphocholine
DPGPC	1, 2-diphytanoyl- <i>sn</i> -glycero-3-phosphocholine
DPPA	dipalmitoyl L-α-phosphatidic acid
DPPC	dipalmitoyl phosphatidylcholine
DPPE	1,2-dipalmitoyl- <i>sn</i> -glycero-3-phosphoethanolamine
DsbB	disulphide bond forming protein B
FBDD	fragment based drug discovery

FID	free induction decay
FPMSMA	4-fluorophenyl)methylsulfanylmethanimidamide
FRAP	fluorescence after photobleaching
GAPS	γ -aminopropylsilane
GPCR	G-protein coupled receptor
GR	1,2,3,9-tetrahydro-3-[(5-methyl-1H-imidazol-4-yl)methyl]-9-(3-amino-(N-fluoresceinthiocarbamoyl)propyl)-4H-carbazol-4-one
HA-hH ₁ R-HIS	heamagglutinin and 6-his tagged human histamine H ₁ receptor
hA ₁ R	human adenosine A ₁ receptor
HTA	ω -hydroxy-undecanethiol
IMAC	immobilized metal affinity chromatography
KcsA	K ⁺ channel from <i>Streptomyces lividans</i>
LB	langmuir–blodgett
LPC	lysophosphatidylcholine
N0840	N ⁶ -cyclopentyl-9-methyladenine
NMR	nuclear magnetic resonance
NTA	nitriolotriacetic acid
OG	octylglucoside
OmpA	outer membrane protein B
PC	phosphatidylcholine
POPC	palmitoyl-oleoyl-phosphatidylcholine
POPG	palmitoyl-oleoyl-phosphatidylglycerol
PBS	phosphate buffered saline
PEEK	polyetheretherketones
PSLB	planar supported lipid bilayers
PWR	plasmon-waveguide resonance
QSAR	quantitative structure activity relationship
SAM	self-assembled monolayer
SCA	scaffold-based classification approach

SDS	sodium dodecyl sulfate
SERIAS	surface-enhanced infrared reflection absorption spectroscopy
SPA	scintillation proximity assay
SPFS	surface plasmon enhanced fluorescence spectroscopy
SPR	surface plasmon resonance
STD	saturation transfer difference
tBLM	tethered bilayer lipid membrane
TIFR	total internal reflection fluorescence microscopy
TINS	target immobilized NMR screening
TMA	tetramethylammonium chloride
TSP	trimethylsilyl-2,2,3,3-tetradeuteriopropionic acid