ENGINEERING CYANOVIRIN-N FOR ENHANCED VIRAL NEUTRALIZATION

Thesis by

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Acknowledgements

I came to Caltech 5 ½ years ago as an enthusiastic first year graduate student ready to tackle the hard problems in protein design and learn computational methods. I quickly realized, however, that there was a reason these problems hadn't been solved before and that I really wasn't much of a computationalist. Luckily, through the good times and the bad, I had an amazing team of supporters who would both listen to me complain endlessly and join in the celebration of successful experiments.

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Abstract

Cyanovirin-N (CVN) is an 11-kDa lectin originally isolated from the cyanobacterium *Nostoc ellipsosporum* during a high-throughput screen for novel anti-HIV activities. In addition to having anti-HIV activity, CVN has since been shown to neutralize a number of other enveloped viruses including influenza and Ebola. This antiviral activity is attributed to two homologous carbohydrate binding sites that specifically bind α(1-2)-linked oligomannose glycosylation sites present on many envelope glycoproteins. Because of its broad ability to neutralize enveloped viruses, CVN is a promising target as a potential therapeutic or prophylactic.

In this work, we oligomerized CVN to determine whether an increase in the number of carbohydrate binding sites has an effect on its viral neutralization activity. To create obligate dimers, we covalently linked multiple copies of CVN through flexible polypeptide linkers. Using HIV-1 as our viral system, we found that a tandem repeat of two CVN molecules (CVN_2) increased the efficacy of HIV-1 neutralization by up to 10-fold. An additional benefit was not seen when CVN was trimerized. We also show here that CVN and the CVN_2 variants show extensive cross-clade reactivity and higher neutralization efficacy than the most broadly reactive neutralizing antibodies. To determine whether any major structural changes or differences in domain swapping occurred because of the linkage, we solved the crystal structures of three dimeric variants and showed that all variants are intramolecularly domain-swapped.

Additionally, we present in this thesis a novel CVN-Fc chimera, a "lectibody," which shows antiviral activity similar to wild-type CVN. This variant is dimerized through the Fc region of an antibody and has the additional benefit of incorporating Fc-

mediated effector functions, which may be therapeutically advantageous. Initial results on the lectibody indicate that domain swapping of CVN has an integral role in the antiviral function as well as in the overall folding and stability of the molecule. Future work on this variant to assay the effector functions as well as create a monodispersive, stable variant are underway.

Although CVN is already a promising candidate for antiviral therapeutics, we show here that engineering CVN to add additional functionalities or creating variants with an increased number of active sites can significantly enhance the potential benefit of these molecules.

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ABBREVIATIONS

BsCM	chorismate mutase from *Bacillus subtilis*
CaM	calmodulin
$CaMKI_p$	calmodulin kinase I peptide
CD	circular dichroism
CV	column volume
CVN/CV-N	cyanovirin-N
CVN_2 LX	cyanovirin-N dimer with a linker length of X amino acids
CVN_3 LX	cyanovirin-N trimer with a linker length of X amino acids
Da	daltons
DTT	dithiothreitol
E. coli	*Escherichia coli*
EcCM	chorismate mutase domain of *E.coli* chorismate mutase-prephenate dehydratase
EDTA	ethylene diamine tetraacetic acid
EGTA	ethylene glycol tetraacetic acid
Fab	antigen binding fragment of an antibody
Fc	constant region of an antibody
FMEC	FASTER-derived minimum energy conformation
GnHCl	guanidine HCl
HERO	hybrid exact rotamer optimization
HIV	human immunodeficiency virus
IC_{50}	concentration at which there is 50% inhibition
IPTG	isopropyl β-D-1-thiogalactopyranoside
k_{cat}	catalytic rate constant
K_D	dissociation binding constant
K_M	Michaelis constant
LB	Luria-Bertani broth
MWCO	molecular weight cut off
NAb	neutralizing antibody
Ni-NTA	nickel-nitrilotriacetic
NMR	nuclear magnetic resonance spectroscopy
OD	optical density at a specific wavelength
ORBIT	optimization of rotamers by iterative techniques
PCR	polymerase chain reaction
PDB	protein data bank
PMSF	phenylmethanesulphonylfluoride (a serine protease inhibitor)
RU	response unit (units for binding on Biacore)
scFv	single chain variable region fragment
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
$smMLCK_p$	smooth muscle light chain kinase peptide
$TCID_{50}$	tissue culture infectious dose 50%
T_m	midpoint of temperature denaturation curve
WT	wild type