Supporting Information

Structural determinants for the interactions of chemically modified nucleic acids with the Stabilin-2 clearance receptor

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Purification of the soluble 190-HARE ecto-domain (s190)

A stable cell line expressing the s190 ecto-domain was previously developed and described in Harris et al[1]. Cells were cultured in 4-chamber Celldisc flasks (Greiner-bio One) at 37°C, 5% CO_2. The s190 protein is secreted from the cells in growth medium containing DMEM supplemented with 8% fetal bovine serum and 50 μg/mL hygromycin B. A batch of 500 mL of condition medium was incubated with 1 mL of packed mAb30 resin. Monoclonal antibody 30 (mAb30) is a monoclonal antibody against rat HARE (175-kDa) isoform that also reacts against the human receptor[2]. The antibody was conjugated with cyanogen-bromide activated sepharose (#C9142, SigmaAldrich) according to the manufacturer's instructions. Both resin and conditioned medium were rotated slowly overnight at 4°C and the resin was separated from the medium using a single gravity flow column (#9704352, BioRad, Hercules, CA, USA). Excess protein and media were washed from the resin using 10 bed volumes of saline (500 mM NaCl, 20 mM sodium phosphate monobasic, pH 7.2). s190 protein was eluted from the resin using four sequential bed volumes of 100 mM glycine, pH 3 which dripped into a 15 mL conical containing 4 bed volumes of 1 M unbuffered Tris buffer, pH 11. The resin was immediately rinsed with saline (150 mM NaCl, pH 7.2) and stored at 4°C for re-use. The eluted protein was concentrated and buffered exchanged with 1X PBS (150 mM NaCl, 20 mM sodium phosphate monobasic) using Vivaspin Turbo 4 concentrators (#VS04T41, MWCO = 100,000, Sartorius) down to a volume of 0.3-0.5 mL and quantified by the Bicinchoninic (BCA) assay (BioRad, Hercules, CA, USA).

Fluorescence polarization assay

Fluorescence polarization experiments were performed using ALEXA647-labeled ASOs synthesized at Integrated DNA Technologies (Coralville, IA, USA). Measurements were performed in 1X phosphate buffered saline (PBS), except for the experiments to determine salt and pH dependence of binding. For those evaluations a 10 mM phosphate buffer with a sodium chloride concentration of 50 to 200 mM and a pH of 5, 6, or 7 was utilized. The assay was set up in 96-well costar plates (black flat-bottomed non-binding) purchased from Corning, NY, USA. Binding was evaluated by adding ALEXA647-labeled ASOs to yield 2 nM concentration to each well containing 100 μL of Stabilin-2 protein from sub nM to low μM concentration. Readings were taken using the Tecan (Baldwin Park, CA, USA) InfiniteM1000 Pro instrument (λ_{ex} =635 nm, λ_{em} =675 nm). Using polarized excitation and emission filters, the instrument measures fluorescence perpendicular to the excitation plane (the 'P-channel') and fluorescence that is parallel to the excitation plane (the 'S-channel'), and then it calculates FP in millipolarization units (mP) as follows: $mP = [(S - P * G) / (S + P * G)] * 1000$. The 'G-factor' is measured by the instrument as a correction for any bias toward the P channel [3]. Polarization values of each ALEXA647-labeled ASO in 1X PBS at 2 nM concentration were subtracted from each measurement. K_d values were calculated with GraphPad Prism 5 software (GraphPad Software, La Jolla, CA, USA) using non-linear regression for curve fit assuming one binding site.

Fig. S1: Amino acid sequence of the s190 ectodomain of Stabilin-2 that was purified and used in the fluorescence polarization assay. Color coding is indicated below.

TKLALFESLPNLLMRLEQMPDYPIFRGYIIQYNLANAIEAADAYTVFAPNNNAIENYIREKKVLSLEEDV
LRYHVVLEEKLLKNDLHNGMHRETMLGFSYFLSFFLHNDQLYVNEAPINYTNVATDKGVIHGLGKVLEIQ
KNRCDNNATTIIRGRCRTCSSELTCPFGTKSLGNEKRRCIYTSYFMGRRTLFIGCQPKCVRTVITRECCA
GFFGPQCQPCPGNAQNVCFGNGICLDGVNGTGVCECGEGFSGTACETCTEGKYGIHCDQACSCVHGRCNQ
GPLGDGSCDCDVGWRGVHCDNATTEDNCNGTCHTSANCLTNSDGTASCKCAAGFQGNGTICTAINACEIS
NGGCSAKADCKRTTPGRRVCTCKAGYTGDGIVCLEINPCLENHGGCDKNAECTQTGPNQAACNCLPAYTG
DGKVCTLINVCLTKNGGCSEFAICNHTGQVERTCTCKPNYIGDGFTCRGSIYQELPKNPKTSQYFFQLQE
HFVKDLVGPGPFTVFAPLSAAFDEEARVKDWDKYGLMPQVLRYHVVACHQLLLENLKLISNATSLQGEPI
VISVSQSTVYINNKAKIISSDIISTNGIVHIIDKLLSPKNLLITPKDNSGRILQNLTTLATNNGYIKFSN
LIQDSGLLSVITDPIHTPVTLFWPTDQALHALPAEQQDFLFNQDNKDKLKEYLKFHVIRDAKVLAVDLPT
STAWKTLQGSELSVKCGAGRDIGDLFLNGQTCRIVQRELLFDLGVAYGIDCLLIDPTLGGRCDTFTTFDA
SGECGSCVNTPSCPRWSKPKGVKQKCLYNLPFKRNLEGCRERCSLVIQIPRCCKGYFGRDCQACPGGPDA
PCNNRGVCLDQYSATGECKCNTGFNGTACEMCWPGRFGPDCLPCGCSDHGQCDDGITGSGQCLCETGWTG
PSCDTQAVLPAVCTPPCSAHATCKENNTCECNLDYEGDGITCTVVDFCKQDNGGCAKVARCSQKGTKVSC
SCQKGYKGDGHSCTEIDPCADGLNGGCHEHATCKMTGPGKHKCECKSHYVGDGLNCEPEQLPIDRCLQDN
GQCHADAKCVDLHFQDTTV*GVFHLRSPLGQYKLTFDKAREACANEAATMATYNQLSYAQKAKYHLCSAGW*
*LETGRVAYPTAFASQNCGSGVVGIVDYGPRPNKSEMWDVFCY*RMKDVNCTCKVGYVGDGFSCSGNLLQVL
MSFPSLTNFLTEVLAYSNSSARGRAFLEHLTDLSIRGTLFVPQNSGLGENETLSGRDIEHHLANVSMFFY
NDLVNGTTLQTRLGSKLLITASQDPLQPTETRFVDGRAILQWDIFASNGIIHVISRPLKAPPAPVKGELG
TELGSEGKPIPNPLLGLDSTRTGHHHHHH

Remnant of the signal sequence from vector plasmid
S190 HARE ectodomain
EGF/EGF-like domains
Fasciclin-1 domains
Link domain
Remnant of the original vector MCS sequence
V5 epitope tag
6xHis tag

References

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2. Harris, E. N.; Weigel, J. A.; Weigel, P. H., (2004) *J Biol Chem 279*, 36201-36209.
3. Goulko, A. A., Zhao, Q., Guthrie, J.W., Zou, H., Le, X.C., Fluorescence Polarization: Recent Bioanalytical Applications, Pitfalls, and Future Trends. In *Standardization and Quality Assurance in Fluorescence Measurements I: Techniques*, Resch-Genger, U., Ed. Springer: Berlin, Heidelberg, 2008; pp 303-322.