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# SLO3 in the fast lane: The latest male contraceptive target with a promising small-molecule inhibitor

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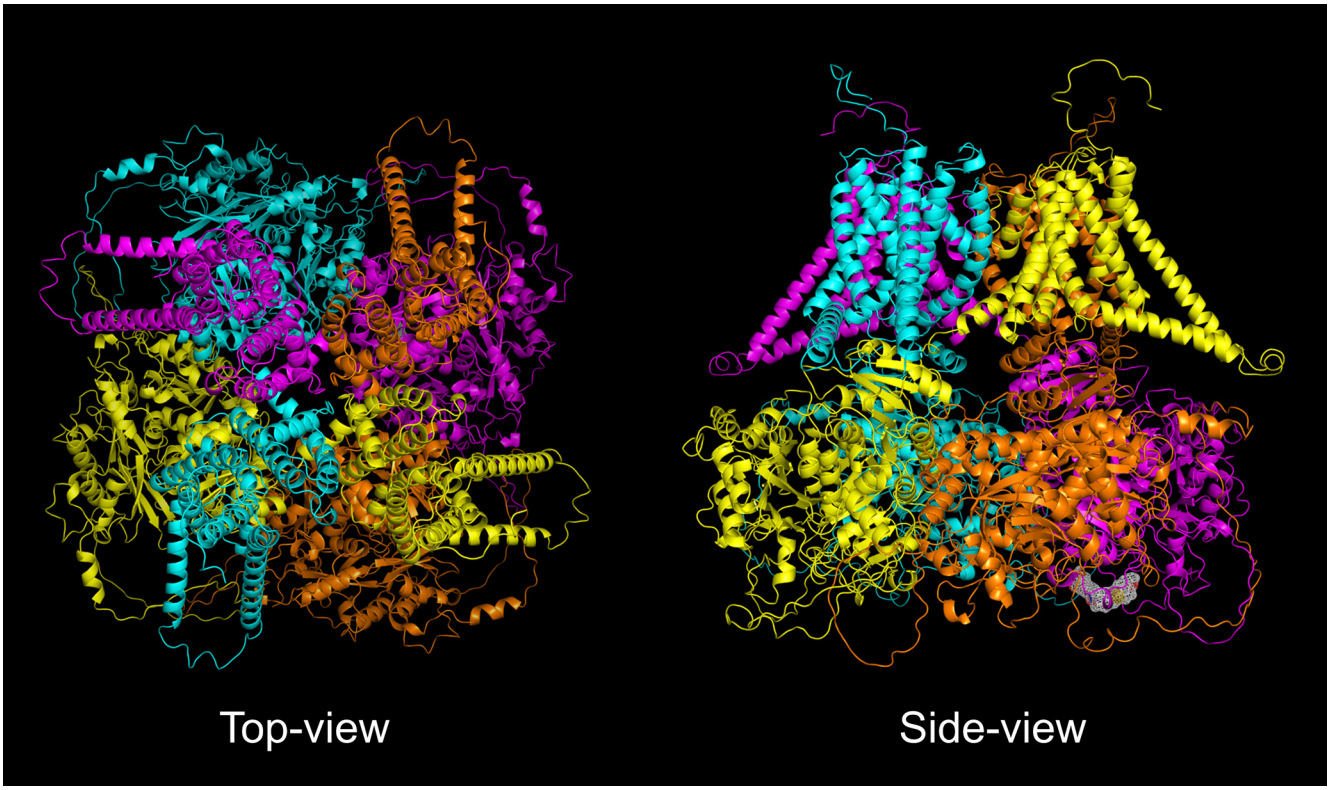


**Fig. 1.** Protein structure model of the SLO3 homotetramer. The SLO3 homotetramer (side view and top view as indicated) was generated by superimposition of SLO3 monomers obtained through AlphaFold (20, 21) with the crystal structure of the highly homologous SLO1 homotetramer (15). The central pore of the SLO3 channel is visible in the top view, while the intramembrane and cytoplasmic regions are visible along the *Top* and *Bottom* halves of the side view, respectively.

Nearly one decade before the first humans set foot on the moon, the birth control pill for women was developed and made available to the public to meet a level of global demand for contraception that many would argue is akin to ameliorating world hunger. Fast forward to more than six decades later, despite an international race to find a male contraceptive pill that has consumed hundreds of millions of dollars, not a single pill for men exists on the market. Part of the reason for this is simple. Hormones act powerfully in the body to influence fertility, and the female pill is a hormonal method. While manipulation of male hormones could be used to cause infertility in men, the side effects have thus far been deemed too dangerous and unsafe to be considered a viable option. Therefore, the quest for a male pill is driven by a relatively unified agreement across the male contraceptive development community that the best drug candidates must target fertility in a nonhormonal manner—or carefully engineered hormonal manner—with perfect to near-perfect efficacy (unwanted pregnancies cannot and must not result when used properly). Furthermore, the best candidate drugs must do so in such a way that they are safe and result in minuscule to no side effects. To achieve this, to ensure safety, many investigators start by looking at druggable protein targets that are present only in the male reproductive tract, or in sperm cells, to help safeguard against effects occurring elsewhere in the body. The next step is to identify drug targets for which genetic studies show that animal models lacking the gene are sterile, not subfertile, to help predict that a

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pronounced contraceptive effect can occur with effective target inhibition. Among the targets and inhibitors that pass these tests, the most desirable ones are those that are reversible in effect and that do not lead to a reduction in testis size or semen volume during use. Only in the last 5 y have a handful of potential drugs emerged, such as EP055 against EPPIN (1), CDD-1102 against BRDT (2), triptonide against JUP/SPEM1 (3), and YCT529 against RARA (4). Now, a new molecule has been added to this short list: VU0546110 (5), a small molecule that inhibits Slowpoke Homolog 3 (SLO3), an ion channel specific to sperm. Because SLO3 channels are absent from women (6), they have the added benefit of being targetable by nonhormonal unisex contraceptives and, therefore, have an easier regulatory pathway to market.

SLO3 is an evolutionarily conserved voltage-gated ion channel that allows outward flow of potassium ions during plasma membrane hyperpolarization in sperm. Expression of the gene encoding SLO3 (*KCNU1*) is specific to the cells that give rise to sperm in mice (7, 8) and humans (9). Deletion of the *Kcnu1* gene in mice results in sterility in males due to abnormal sperm physiology with no overtly abnormal nonreproductive phenotypes detected (10). Spermatogenesis and testicular function are spared in mutant males, while capacitating sperm are depolarized at baseline and display impaired hyperpolarization and motility, a bent "hairpin" shape, and failure to undergo the acrosome reaction (10, 11). All of these features make SLO3 a desirable male contraceptive drug target.

**In Lyon et al., the authors identify VU0546110, a potent small-molecule inhibitor of SLO3, through screening of a chemical library of 50,240 compounds.**

Lyon et al. (5) identify VU0546110, a potent small-molecule inhibitor of SLO3, through screening of a chemical library of 50,240 compounds (5). They show that VU0546110 exerts its effect on human sperm by blocking SLO3 and inhibiting hyperpolarization (5). Based on IC50 values reported by Lyon et al., VU0546110 has 46-fold selectivity to SLO3 over Slowpoke Homolog 1 (SLO1; encoded by the *KCNMA1* gene), its closest paralog with ~40% sequence similarity. Although quinidine and quinine were previously reported to inhibit SLO3 (12), causing changes to sperm motility (12), neither of these molecules are selective to SLO3. This makes their use as a contraceptive problematic, as they could bind to and inhibit SLO1 at certain doses (12). Although SLO1 does not appear to be expressed in late-stage human spermatids (6), since it is expressed in the brain, prostate, gallbladder, and other organs (6) with several known channelopathies caused by loss of channel function (13), this suggests that the use of quinidine and quinine as contraceptives could lead to significant off-target side effects in men (14). Thus, identification of VU0546110 as a highly specific inhibitor for SLO3 is significant as its selectivity for SLO3 over SLO1 reduces the potential for off-target side effects in men. Further, VU0546110 can also be a novel "tool compound" with which to better understand the mechanism and role of SLO3 in sperm physiology and male fertility.

Unlike its paralog SLO1, SLO3 is regulated by intracellular pH. Alkalization inside the cell activates SLO3 (10, 15). SLO1 and SLO3 share very similar architecture and both channels form homotetramers in the membrane (16). The protein structure model for SLO3 homotetramer, showing the pore, intramembrane, and cytoplasmic regions, is presented in Fig. 1. Each protomer is formed by seven transmembrane helices (S0 to S6) with S1 to S4 responsible for voltage sensing and S5 and S6 forming the pore for ion flow (17). S6 of each protomer is connected to a cytosolic domain (CTD) consisting of two regulators of potassium conductance (RCK) domains (18), which are responsible for the modulation of SLO family channels by different intracellular ions (19). Although the binding site and interactive residues of VU0546110 were not reported by Lyon et al., it is possible that the specificity of VU0546110 for SLO3 over SLO1 is achieved by targeting the CTD of SLO3. This view is supported by the ClogP value of VU0546110 (ClogP = 3.362), which indicates that the molecule has a propensity to traverse the cell membrane while remaining hydrophilic. Follow-up studies to characterize the binding pocket and binding pose of VU0546110 will provide a better understanding of how this compound exerts its effect. Without these data, it is equally possible that VU0546110 exerts its effects by blocking the pore or by interfering with channel opening from the outside of the cell. From the perspective of drug development, VU0546110 is a hit compound obtained through a high-throughput screen. Further optimization of VU0546110 through medicinal chemistry studies will likely yield derivatives that are more potent, selective, or that have more desirable traits such as bioavailability.

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