Electronic Supplementary Material (ESI) for Chemical Science.
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1. Supplemental Data

Table S1. Retention factors for testosterone on immobilized HSA and control columns in the presence of various mobile phases[a].

Mobile phase	Retention factor on HSA column[b]	Retention factor on control column[b]	Retention factor due to HSA[c]
67 mM phosphate buffer (KPB, pH 7.4)	26.6 (± 4.0)	4.4 (± 0.7)	22.2 (± 4.1)
KPB (pH 7.4) + 40 µM citrate	16.7 (± 0.9)	5.9 (± 0.1)	10.8 (± 1.1)
KPB (pH 7.4) + 20% DMSO	14.2 (± 1.2)	4.0 (± 0.3)	10.2 (± 1.2)

[a]All values in parentheses are a range of ± 1 S.D. (n = 3-4).

[b]The retention factor (k) on the HSA column or control column was calculated by using the relationship $k = (t_R - t_M)/t_M$, where t_R is the retention time for testosterone on the column and t_M is the column void time (e.g., as measured by using sodium nitrate).

[c]The retention factor for testosterone due to only HSA was found by calculating the difference between the retention factors that were measured on the HSA column and the control column.

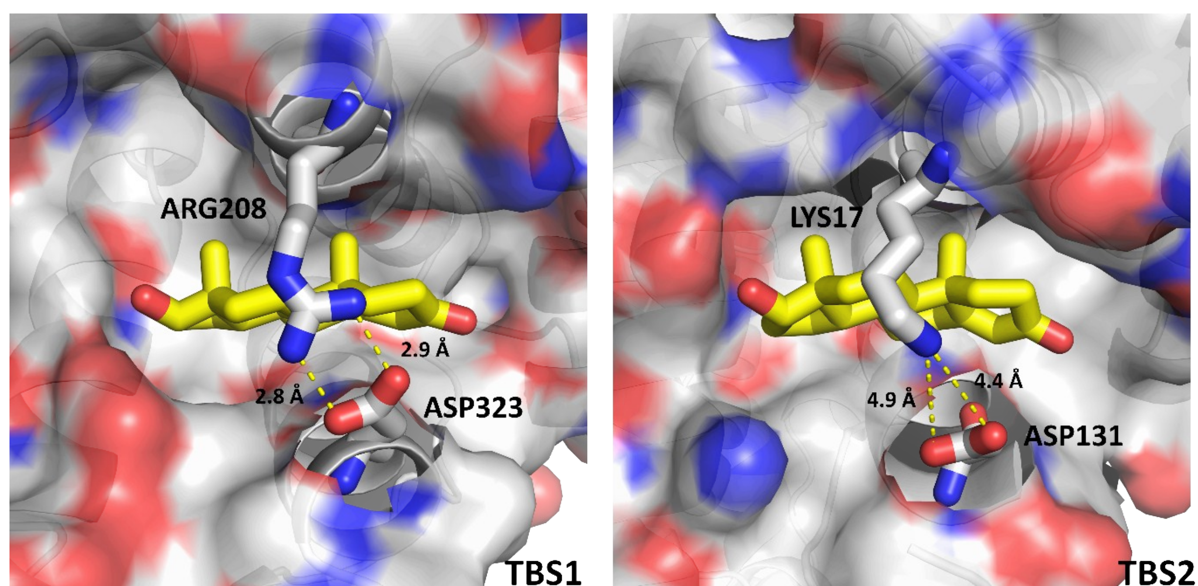

Figure S1. Testosterone binding sites are protected by non-covalent interactions between Arg208 and Asp323 in TBS1 and Lys17 and Asp131 in TBS2. The color of the protein surface indicates the character of the environment, and the color scheme is as follows: gray for carbon atoms in ESA, red for oxygen atoms, and blue for nitrogen atoms. Testosterone's carbon atoms are shown in yellow.

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ESA   1    DTHKSEIAHRFNDLGEKHFKGLVLVAFSQYLQQCPFEDHVKLVNEVTEFAKKCAADESAE   60
HSA   1    DAHKSEVAHRFKDLGEENFKALVLIAFAQYLQQCPFEDHVKLVNEVTEFAKTCVADESAE   60
           *:****:****:****::**.***:**:***********************.*.******

ESA  61    NCDKSLHTLFGDKLCTVATLRATYGELADCCEKQEPERNECFLTHKDDHPNLPKL-KPEP   119
HSA  61    NCDKSLHTLFGDKLCTVATLRETYGEMADCCAKQEPERNECFLQHKDDNPNLPRLVRPEV   120
           ********************* ****:**** *********** ****:****:* :** 

ESA 120    DAQCAAFQEDPDKFLGKYLYEVARRHPYFYGPELLFHAEEYKADFTECCPADDKLACLIP   179
HSA 121    DVMCTAFHDNEETFLKKYLYEIARRHPYFYAPELLFFAKRYKAAFTECCQAADKAACLLP   180
           *. *:**::: :.** *****:********.*****.*:.*** ***** * ** ***:*

ESA 180    KLDALKERILLSSAKERLKCSSFQNFGERAVKAWSVARLSQKFPKADFAEVSKIVTDLTK   239
HSA 181    KLDELRDEGKASSAKQRLKCASLQKFGERAFKAWAVARLSQRFPKAEFAEVSKLVTDLTK   240
           *** *::.   ****:****:*:*:*****.***:******:****:******:******

ESA 240    VHKECCHGDLLECADDRADLAKYICEHQDSISGKLKACCDKPLLQKSHCIAEVKEDDLPS   299
HSA 241    VHTECCHGDLLECADDRADLAKYICENQDSISSKLKECCEKPLLEKSHCIAEVENDEMPA   300
           **.***********************:*****.*** **:****:********::*::*:

ESA 300    DLPALAADFAEDKEICKHYKDAKDVFLGTFLYEYSRRHPDYSVSLLLRIAKTYEATLEKC   359
HSA 301    DLPSLAADFVESKDVCKNYAEAKDVFLGMFLYEYARRHPDYSVVLLLRLAKTYETTLEKC   360
           ***:*****.*.*::**:* :******* *****:******** ****:*****:*****

ESA 360    CAEADPPACYRTVFDQFTPLVEEPKSLVKKNCDLFEEVGEYDFQNALIVRYTKKAPQVST   419
HSA 361    CAAADPHECYAKVFDEFKPLVEEPQNLIKQNCELFEQLGEYKFQNALLVRYTKKVPQVST   420
           ** ***  ** .***:*.******:.*:*:**:***::***.*****:******.*****

ESA 420    PTLVEIGRTLGKVGSRCCKLPESERLPCSENHLALALNRLCVLHEKTPVSEKITKCCTDS   479
HSA 421    PTLVEVSRNLGKVGSKCCKHPEAKRMPCAEDYLSVVLNQLCVLHEKTPVSDRVTKCCTES   480
           *****:.*.******:*** **::*:**:*::*::.**:***********:::*****:*

ESA 480    LAERRPCFSALELDEGYVPKEFKAETFTFHADICTLPEDEKQIKKQSALAELVKHKPKAT   539
HSA 481    LVNRRPCFSALEVDETYVPKEFNAETFTFHADICTLSEKERQIKKQTALVELVKHKPKAT   540
           *.:*********:** ******:************* *.*:*****:**.**********

ESA 540    KEQLKTVLGNFSAFVAKCCGREDKEACFAEEGPKLVASSQLALA-                583
HSA 541    KEQLKAVMDDFAAFVEKCCKADDKETCFAEEGKKLVAASQAALGL                585
           *****:*:.:*:*** ***   :***:****** ****:** **.
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Figure S2. Alignment of ESA and HSA sequences. Residues involved in binding of testosterone molecules in ESA (according to PISA server calculations) and analogous residues inferred for HSA are marked in red (TBS1) and blue (TBS2).

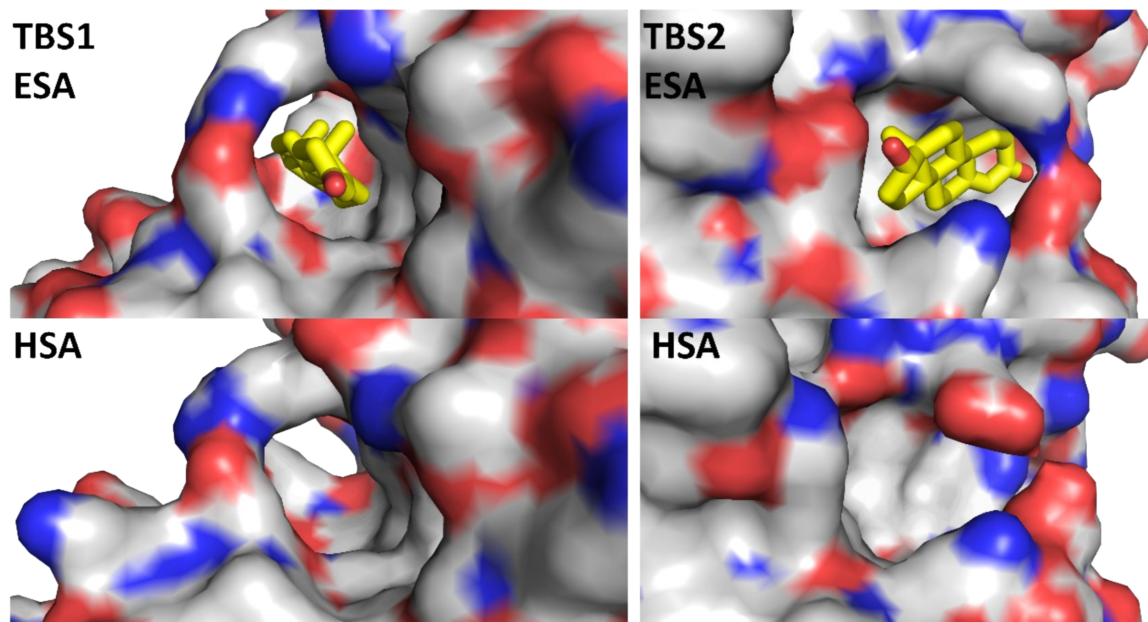

Figure S3. Comparison of environment's character of TBS1 and TBS2 in ESA and HSA. The color of the protein surface indicates the character of the environment, and the color scheme is as follows: gray for carbon atoms in ESA and HSA, red for oxygen atoms, and blue for nitrogen atoms. Testosterone's carbon atoms are shown in yellow.