**Supporting Information**

# Elucidation of New Binding Interactions with the Human Tsg101 Protein Using Modified HIV-1 Gag-p6 Derived Peptide Ligands

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## I. X-ray Crystallography

**Table SI-1**. Statistics of data collection and crystallographic refinement for the structures of peptides **2** and **3** complexed to Tsg101 protein.

| Crystal | Peptide 2 | Peptide 3 |
|---|---|---|
| Constructs | TSG101 (2-146)<br>43-38 GG | TSG101 (2-146)<br>43-38 GG |
| Space group, unit cell | $P2_12_12_1$<br>a = 33.6 Å, b = 45.8 Å,<br>c = 85.4 Å | $P2_12_12_1$<br>a = 33.6 Å, b = 45.7 Å,<br>c = 88.5 Å |
| X-ray source | CuKα | CuKα |
| Wavelength (Å) | 1.5493 | 1.5493 |
| Resolution (Å) (last shell) | 1.8 (1.80 – 1.83) | 1.8 (1.80 – 1.83) |
| No. of unique reflections | 12869 | 12870 |
| I/σ(I)σ (last shell) | 38.4 (5.6) | 37.0 (5.9) |
| Rsym (%) | 7.2 (33.4) | 7.2 (40.0) |
| Data completeness (%) | 99.4 (95.1) | 97.5 (95.3) |
| **Refinement** | | |
| R factor (%) | 21.0 (28.8) | 19.8 (24.9) |
| Free R factor (%) | 25.1 (28.2) | 24.1 (30.9) |
| R.m.s. bond length (Å) | 0.005 | 0.005 |
| R.m.s. bond angle (°) | 1.3 | 1.3 |
| Average B value (Å$^2$) | 28.3 | 21.6 |
| Number of atoms | protein 1199<br>water 123 | protein 1193<br>water 126 |

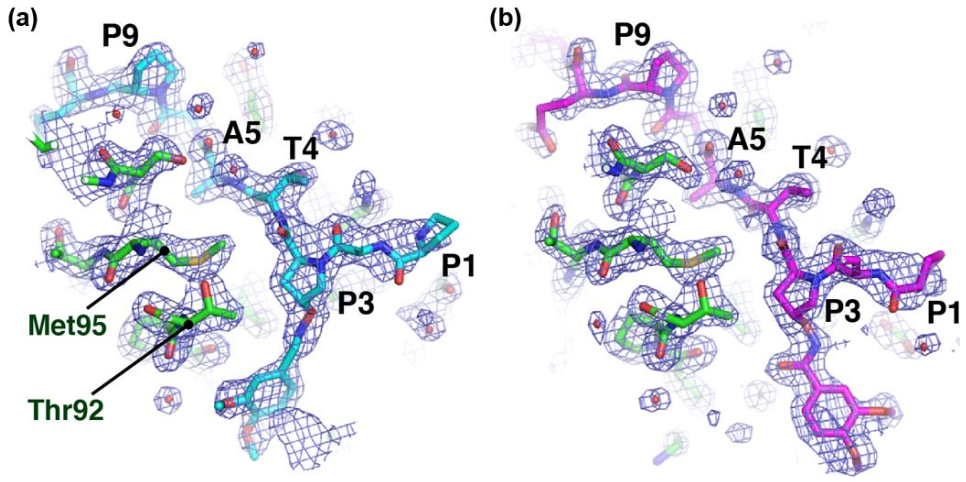



**Figure SI-1**. 1.8 Å 2Fo-Fc electron density maps of Tsg101 – bound peptide **2** (a) and **3** (b) superimposed with final models.

**II. Tsg101 Binding Assays.** A complex of 10 μM Tsg101 and **SI-1** (5 μM) (see Figure SI-2) in 50 mM Hepes pH 7.5, 150 mM NaCl, 100 μM TCEP and 5 mM mercaptoethanol was placed in a 96 well Costar polypropylene plates (Corning, NY). Unlabeled peptides (**2**, **5** – **7**) were serially diluted into the complex with starting concentrations of 500 μM and 40 μL was removed and transferred into 384-well Costar polypropylene plates (Corning, NY) and incubated at room temperature (30 minutes). Samples were excited at 485 nm and the emission intensities at 535 nm from the parallel and perpendicular planes were measured using a Tecan Ultra plate reader (Durham, NC). Binding data is presented in Table 1 of the main text.

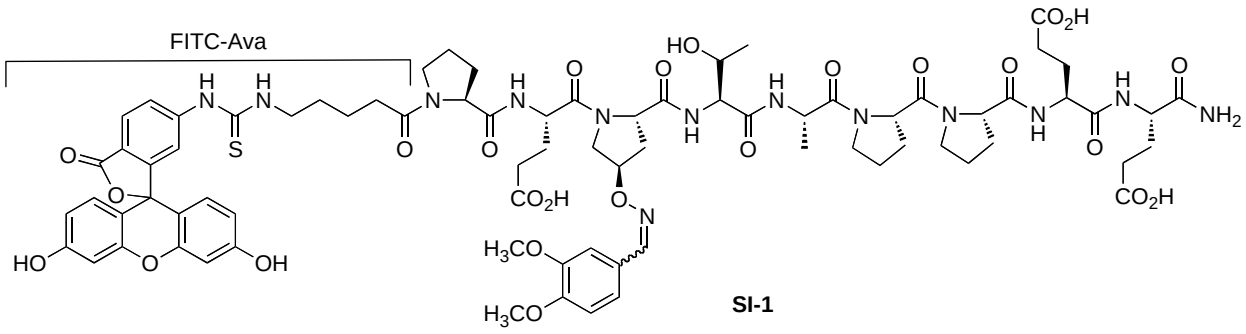



**Figure SI-2**. Structure of **SI-1**.

**Determination of Ki Values.** Ki values (Table 1) were derived from fluorescence anisotropy data by assuming a simple competitive inhibition model described through a set of conservation equations. A non-linear description was applied to all peptides in an experimental series in a global analysis. A maximum likelihood non-linear mixed effects system (fitting system) was used to estimate the equilibrium binding constants ($K_i$) [Statistics Toolbox - MATLAB a from: http://www.mathworks.com/products/statistics/ ]. Anisotropy results were transformed to fractional binding as part of the fitting system. This transformation required estimating the minimum and maximum anisotropy values for each titration. These values were estimated in the fitting system as random effects. A single $K_d$ value was estimated and assumed to be constant through out each set of experiments. Each $K_i$ value was estimated as a fixed effect in this fitting system.

**Application of Surface Plasmon Resonance (SPR) to Measure the Ability of Peptides to Inhibit Tsg101 – p6 Binding**. GST and GST-p6 were captured on an anti-GST antibody chip, then 10 mM of Tsg101 protein either alone or in the presence of increasing concentrations of peptide **2** or peptide **6** were injected over the sensor chip surface. The amount of Tsg101 protein bound to the p6 protein was measured with increasing concentrations of peptides. Experiments were performed in duplicate. Data from one run for each peptide are shown in Figure SI-3.

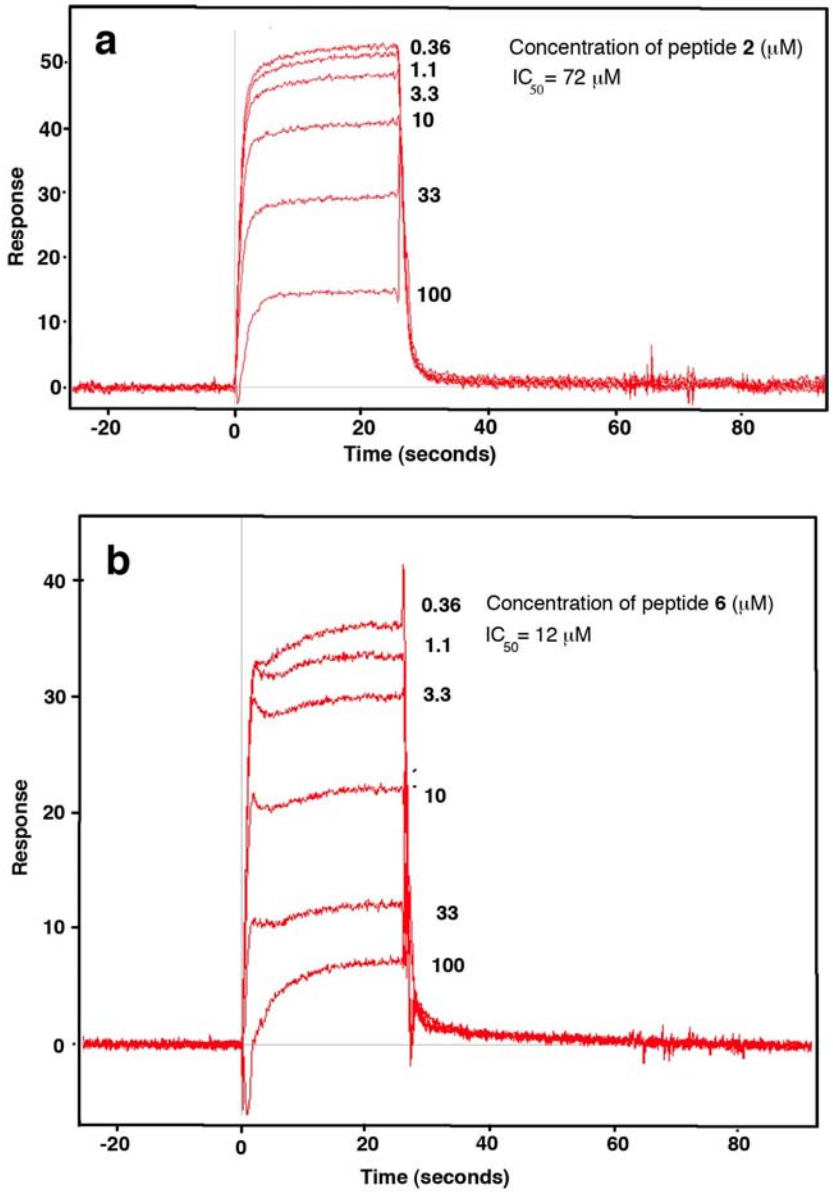
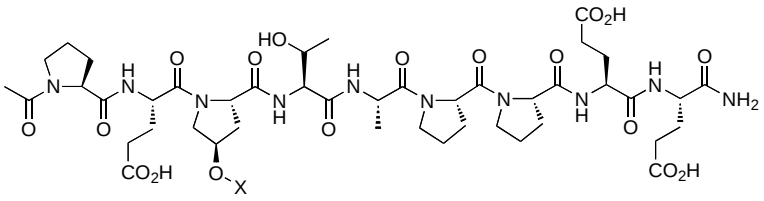



**Figure SI-3**. SPR data for the binding of Tsg101 in solution to chip-bound p6 protein in the presence of increasing concentrations of competing peptides. (a) Peptide **2**; (b) Peptide **6**. Indicated $IC_{50}$ values were determined from the data using a four parameter logistical function.

## III. Synthetic

**Synthesis of Peptide SI-2.** Peptide **SI-2** (Figure SI-4) was prepared as previously described [Liu, F.; Stephen, A. G.; Fisher, R. J.; Burke, T. R. Protected aminooxyprolines for expedited library synthesis: Application to Tsg101-directed proline-oxime containing peptides. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1096-1101]. In summary, solid-phase synthesis was conducted on NovaSyn®TGR resin (purchased from Novabiochem, cat. no. 01-64-0060) using standard Fmoc solid-phase protocols. Active ester coupling was employed using 1-hydroxybenzotriazole (HOBT) and *N, N'*-diisopropylcarbodiimide (DIC) (5 equivalents: for primary amines, single coupling, 2 h and for secondary amines, double coupling, 5 equivalents, 4 h). Amino terminal acetylation was by using *N*-acetylimidazole (10 equivalents in DMF, 7 h). Introduction of the (4*R*)-aminooxyproline residue was achieved using *N*-Fmoc (2S, 4R)-[4-(Boc-amino)oxy] Finished resins were washed with DMF, dichloromethane, ethyl acetate and diethyl ether and then dried. The peptide was cleaved from the resin by treatment with 5 mL of trifluoroacetic acid (TFA) : triisopropylsilane (TIS) : $H_2O$ (90 : 5 : 5, 4 h). Resin was removed by filtration, washed with TFA (3 x) and the filtrate was concentrated *in vacuo*, and crude peptide was precipitated by the addition of diethyl ether and the precipitate was washed with diethyl ether (2 x). The resulting solid was dissolved in aqueous acetonitrile and purified by reverse phase preparative HPLC using a Phenomenex $C_{18}$ column (21.20 mm dia x 250 mm) with a linear gradient from 0% aqueous acetonitrile (0.1% trifluoroacetic acid) to 100% acetonitrile (0.1% trifluoroacetic acid) over 40 minutes at a flow rate of 10.0 mL/minute, with detection at 220 and 254nm. Lyophilization provided the product as a white powder.

**Synthesis of Oxime Peptide Derivatives 5 - 7.** To HPLC-purified aminooxy-containing parent peptide **SI-2** (15 μM) in DMSO (10 μL) was added the appropriate aldehydes (15 μM) in DMSO (10 μL) along with acetic acid (70 μM) in DMSO (10 μL) and the mixtures were gently agitated at room temperature (over night) to provide oxime products in sufficient purity (greater than 90% by HPLC) for direct determination of Tsg101-binding affinities as described above in Section II. Purification by HPLC provided oxime peptides **5 – 7** as white solids.



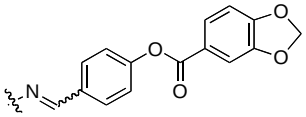
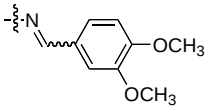
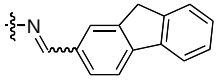
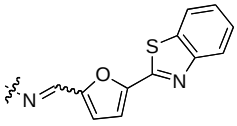
| No | X | No | X |
|---|---|---|---|
| **SI-2**[a] |  | **6** |  |
| **2**[a] |  | **7** |  |
| **5** |  | | |

**Figure SI-4**. Structures of peptides described in the Synthetic Section. ([a]Previously reported; *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1096-1101.)