LIGAND BINDING TO ZN^{2+} BINDING PROTEINS

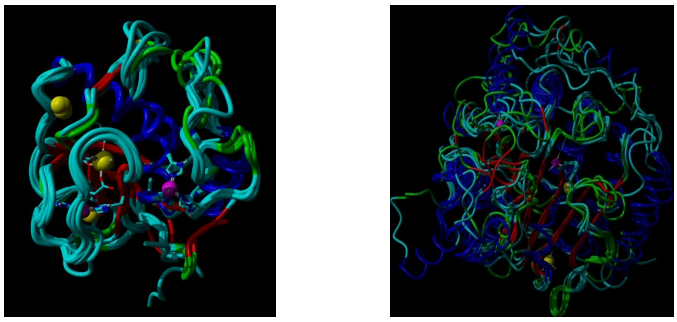
A large percentage of enzyme targets have metal ions, such as Mg^{2+}, Ca^{2+}, and Zn^{2+}, in their active sites that play an important role in substrate binding and catalysis.
Among them there are Matrix Metallo Proteases (MMP) and Histone Deacetylases (HDAC). MMPs are a family of zinc-dependent neutral endopeptidases collectively capable of degrading essentially all matrix components. This family, which includes well over 20 metalloproteinases has been implicated in inflammatory diseases such as chronic obstructive airway diseases, atherosclerosis, bone degenerative conditions including rheumatoid arthritis and osteoporosis, wound repair and, perhaps most well known, cancer. MMPs are dramatically up-regulated in malignant tissues and play a role in both metastasis and angiogenesis. Consequently over 20 MMP modulating agents are in pharmaceutical development, almost half of which are indicated for cancer.
Modulation of the acetylation state of histones plays a pivotal role in the regulation of gene expression.
Histone deacetylases (HDACs) catalyze the removal of acetyl groups from lysines near the N termini of histones.
Several inhibitors of HDACs (HDIs) have been shown to exert antitumor effects. Interestingly, some of the HDIs exerted a broad spectrum of antiprotozoal activity.
Moreover histone acetylation status is a key factor in the regulation of inflammatory gene transcription and HDACs are potential drug targets for inflammatory diseases.
Knowledge of the structure and of the flexibility of loops and side chains surrounding the catalytic binding site is very important for designing new inhibitors against MMPs and HDACs.
However docking compounds into metal-containing active sites is a very challenging task because of the multiple coordination geometries and the lack of sufficiently accurate force field parameters for the ligand-metal interactions. These conditions may alter the hydration and the protonation state of residues, such as Asp, Glu, Arg, Lys, and His in the active site, both factors that are difficult to determine directly from crystal structures.
S.A.F.AN. BIOINFORMATICS developed a procedure able to correctly rate the binding of inhibitors to Zn^{2+} containing binding sites.
The procedure is based on the Lie method (Åqvist et. al., Prot Eng (1994);7:385-391) coupled to a dihedral sampling protocol.
MMP catalytic domains are highly homologous and, as Figure 1 shows, their three dimensional structures are well superimposable.

In order to validate our method, we tested its ability to rate the binding of the same compound to different MMPs and of different compounds to the same MMP. GM6001 MMP inhibitor, also known as Ilomastat or N-[(2R)-2-(hydroxamidocarbonylmethyl)-4-methylpentanoyl]-L-tryptophan methylamide, is a potent, broad spectrum inhibitor of collagenases. Experimental Kis of complexes between GM6001 and MMP2, MMP3, and MMP8 are known and are shown in Table 1 alongside the binding energies computed using the S.A.F.AN. BIOINFORMATICS method.

	MMP2	MMP8
S.A.F.AN. (kJ/mol)	90,6	90,3
experimental K_i (nM)	0,5	0,1

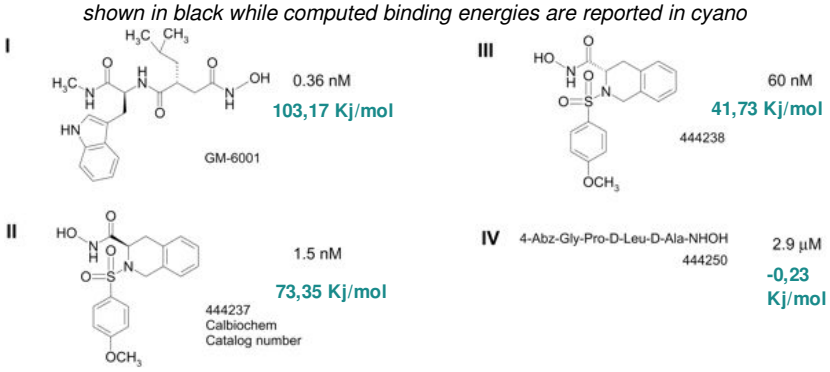
Table 1: Comparison of GM6001:MMP experimental and computed binding energies in which more positive values indicates stronger binding

Figure 1: Superimposition of MMP (on the left) and HDAC (on the right) three dimensional structures. Zn2+ ions are shown in magenta and Ca2+ atoms are shown in yellow



MMP-26 (endometase/matrilysin-2) is the smallest member of the MMP family, comprised of only pro- and catalytic domains. MMP-26 promotes the invasion of a highly metastatic and tumorigenic prostate cancer cell line through its activation of proMMP-9. The article published by Park et al. 2002 *J. biol. chem.* **277**, 35168–35175 reports experimental K_i for MMP26 complexed to 4 different inhibitors. Figure2 reports the chemical structures of those inhibitors alongside with their experimental K_i and the binding energies computed using S.A.F.AN. BIOINFORMATICS method.

Figure 2: MMP-26 Inhibitors: The experimental apparent inhibition constants are shown in black while computed binding energies are reported in cyano



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