# Combination of *in silico* Approaches to Identify Fluorescent Probes Preventing $PrP^{Sc}$ Replication in Prion Diseases

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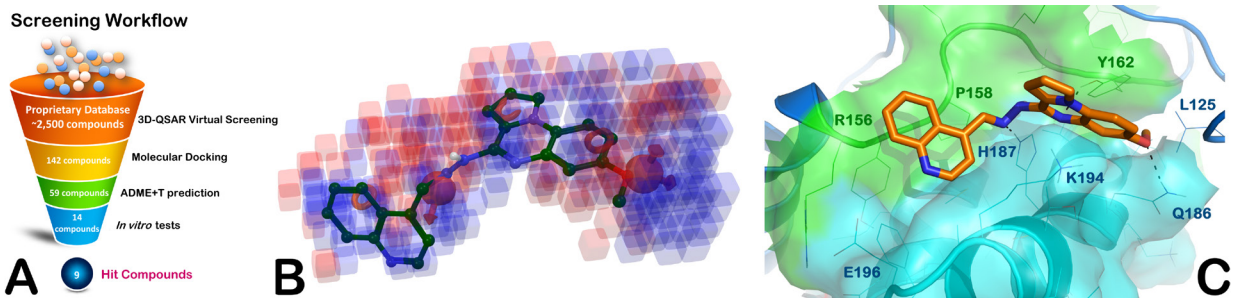
Prion diseases are neurodegenerative disorders caused by the accumulation of the misfolded protein $PrP^{Sc}$ (pathological variant of the cellular protein $PrP^{C}$). Since, no treatments for these diseases are available; the discovery of diagnostic tools and specific therapeutics is urgently required. [1] It was established that molecules that bind $PrP^{C}$ can prevent its misfolding, arresting the progression of disorders related to the abnormal PrP protein. So, *in silico* methods represent a valuable source to discover molecules with desired properties. Based on our knowledge in virtual screening, we designed a workflow to select molecules preventing $PrP^{C}$ misfolding. Phase software (Schrödinger, LLC, New York, NY) was used to derive a 3D-QSAR model, using pharmacophore-based alignment, coupled to molecular docking and physico-chemical properties prediction for identifying molecules able to inhibit the misfolding of $PrP^{C}$ (Fig. A).




The pharmacophore model (AARRR.20), built using 9 highly active compounds, was used as alignment rule for deriving a 3D-QSAR model (Fig. B) considering 58 molecules spanning five orders of magnitude (including 9 highly active compounds). These selected molecules, interacting with the same binding site (D-pocket), effectively inhibit the misfolding process. The model was firstly validated *in silico*, by a decoys set, evaluating the Güner and Henry score (*GH*) and the Enrichment Factor (*EF*), and by using the ROC curve analysis. Next, the 3D-QSAR model was experimentally validated. An *in silico* proprietary database screening (>2,500 compounds) was executed to discover new scaffolds with anti-prion properties. Subsequently, a docking study using Glide software (Schrödinger, LLC, New York, NY) against D-pocket was performed. Finally, the resulting hits were analyzed by means of QikProp (Schrödinger, LLC, New York, NY) and by FAFDrugs3.0 to avoid molecules which behave as Pan Assay Interference Compounds (PAINS). The selected hits (14 compounds) were biologically evaluated to confirm our *in silico* approach. Gratifyingly, 9 out of 14 retrieved hits, characterized by low toxicity, inhibited $PrP^{Sc}$ accumulation in prion-infected neuroblastoma cells (ScN2a). Among them, the pyrroloquinoxalinehydrazone (Fig. C) showed higher potency ($IC_{50}$=1.6 μM). This molecule also binds to $PrP^{Sc}$ aggregates in infected ScN2a cells with a fluorescence pattern similar to that found for Thioflavin-T. [2] By using the described protocol we identified theranostics preventing the pathological transition of $PrP^{C}$ to $PrP^{Sc}$. The combination of the anti-prion profile with a fluorescence imaging behavior and the brain permeability suggests the hit as a prototypic tool for the development of diagnostic and therapeutic probes for prion diseases.

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[1] S.B. Prusiner, *Science*, **2012**, *336*, 1511-1513.

[2] L. Zaccagnini, S. Brogi, *et al.*, *Eur. J. Med. Chem.*, **2017**, *127*, 859-873.