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Article

Designing Nerve Growth Factor Analogues to Suppress Pain Signal Transduction Mediated by the p75NTR-NGF-TrkA Complex: A Structural and Biophysical Perspective

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Abstract: As a key mediator of chronic pain, neurotrophin nerve growth factor (NGF) binds to two neurotrophin receptors: p75 neurotrophin receptor (p75NTR) and tyrosine kinase receptor A (TrkA). The formation of the p75NTR-NGF-TrkA complex is implicated in the potentiation of chronic pain signals, making it an attractive target for therapeutic explorations. NGF analogues and monoclonal antibodies (mAbs) targeting NGF represent two distinct approaches in modulating the intricate signaling pathways involved in pain transduction mediated by the p75NTR-NGF-TrkA complex. While NGF analogues offer the advantage of tailored design to fine-tune neurotrophic responses, monoclonal antibodies provide a more systemic and comprehensive blockade of NGF, inhibiting its interactions with both p75NTR and TrkA receptors. However, the use of mAbs may pose challenges related to potential side effects and interference with the physiological functions of NGF. As a result, balancing the benefits and drawbacks of the two approaches is critical for advancing therapeutic strategies towards the alleviation of p75NTR-NGF-TrkA-mediated pain. In this study, therefore, a novel structural and biophysical approach was employed for the design of NGF analogues to suppress p75NTR-NGF-TrkA-related chronic pain signaling. Employing high-throughput structural modeling and biophysics-based intermolecular binding affinity calculations, this article for the first time puts forward a set of NGF analogues, including in particular NGF analogues with four site-specific mutations, for whose dimerizations the K_d at 37 °C were reduced by three orders of magnitude (from 10^{-9} M to 10^{-6} M) compared to the K_d at 37 °C for the dimerization of native NGFs. Overall, the integration of structural and biophysical perspectives enhances our understanding of the rational design of NGF analogues as promising candidates for the development of NGF-targeted analgesic therapies, which balances the benefits and drawbacks of anti-NGF antibodies and NGF analogues for advancing therapeutic strategies towards the alleviation of p75NTR-NGF-TrkA-mediated pain.

Keywords: Nerve growth factor (NGF); NGF analogues; anti-NGF antibodies; chronic pain signal transduction; p75NTR-NGF-TrkA complex;

1. Introduction

Chronic pain imposes a substantial burden on global healthcare systems, affecting millions of individuals and severely compromising their quality of life [1–4]. Unraveling the intricate mechanisms underlying pain signal transduction is essential for developing targeted and effective therapeutic interventions [5–7]. The p75 neurotrophin receptor (p75NTR)-nerve growth factor (NGF)-tyrosine kinase receptor A (TrkA) complex has emerged as a critical nexus in the modulation of nociceptive signaling, presenting a promising target for innovative analgesic strategies [8–11].

Historically, nerve growth factor (NGF) was first discovered approximately 60 years ago by Rita Levi-Montalcini as a protein that induces the growth of nerves [12–15]. It is now known that NGF is also associated with Alzheimer's disease and intractable pain, and hence, it, along with its high-affinity receptor, tropomyosin receptor kinase (Trk) A, is considered to be 1 of the new targets for therapies

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being developed to treat these diseases [16–22]. Anti-NGF antibody and TrkA inhibitors are known drugs that suppress NGF/TrkA signaling, and many drugs of these classes have been developed thus far. Interestingly, local anesthetics also possess TrkA inhibitory effects. This manuscript describes the development of an analgesic that suppresses NGF/TrkA signaling, which is anticipated to be one of the new methods to treat intractable pain [23–28].

While the implication of NGF and its receptors, TrkA and p75NTR (referred to as the NGF system) in CNS autoimmune neuroinflammation is not fully elucidated, the necessity of designing nerve growth factor (NGF) analogues to suppress pain signal transduction mediated by the p75NTR-NGF-TrkA complex arises from the intricate and multifaceted nature of the neurotrophic signaling pathways involved in CNS autoimmune neuroinflammation [29–34]. Since native NGF plays a pivotal role in both promoting neuronal survival and contributing to pain sensitization through its interactions with the p75NTR and TrkA receptors, the delicate balance between these contrasting effects underscores the need for targeted interventions that selectively modulate the signaling cascade to alleviate pain without compromising essential neurotrophic functions [35–38]. Therefore, in this article, designing NGF analogues provides a strategic approach to fine-tune the molecular interactions within the p75NTR-NGF-TrkA complex, aiming to attenuate the nociceptive signals while preserving the beneficial aspects of NGF-mediated neurotrophic support [39–46].

2. Materials and Methods

According to a structure search of the Protein Data Bank (PDB) [47], as of March 28, 2024, there are a total of sixteen experimentally determined NGF-related structures deposited into the PDB, as listed in Table 1.

PDB ID	Structure Title (release date from newest to oldest)
8DWN	Crystal structure of bis-phosphorylated insulin receptor kinase domain
6PL1	TRK-A IN COMPLEX WITH LIGAND 1B
6NPT	TRK-A IN COMPLEX WITH LIGAND 1
6NSP	TRK-A IN COMPLEX WITH LIGAND 9
6NSS	TRK-A IN COMPLEX WITH LIGAND 6
5WR7	Crystal structure of Trk-A complexed with a selective inhibitor CH7057288
4XPJ	Crystal structure of Nerve growth factor in complex with lysophosphatidylinositol
4NWT	Crystal structure of the anti-human NGF Fab APE1531
4NWU	Crystal structure of APE1551, an anti-human NGF Fab with a nine amino acid insertion in CDR H1
2LPN	Solution Structure of N-Terminal domain of human Conserved Dopamine Neurotrophic Factor (CDNF)
4EFV	Crystal structure of OIF from Llama seminal plasma
2IFG	Structure of the extracellular segment of human TRKA in complex with nerve growth factor
1SG1	Crystal Structure of the Receptor-Ligand Complex between Nerve Growth Factor and the Common Neurotrophin Receptor p75
1HE7	Human Nerve growth factor receptor TrkA
1WWW	NGF IN COMPLEX WITH DOMAIN 5 OF THE TRKA RECEPTOR
1BTG	CRYSTAL STRUCTURE OF BETA NERVE GROWTH FACTOR AT 2.5 A RESOLUTION IN C2 SPACE GROUP WITH ZN IONS BOUND

Table 1. Experimentally determined NGF-related structures (released newest from oldest) in the Protein Data Bank (PDB [47]) as of March 28, 2024, QUERY code: Additional Structure Keywords HAS EXACT PHRASE "NERVE, GROWTH FACTOR".

Among the sixteen, PDB ID **2IFG** [48] is the only experimental structure of the extracellular segment of human TRKA in complex with nerve growth factor, as shown by Figure 1. Therefore, in this article, PDB ID **2IFG** [48] is chosen here as a structural template for the design of NGF analogues to suppress pain signal transduction mediated by the p75NTR-NGF-TrkA complex.

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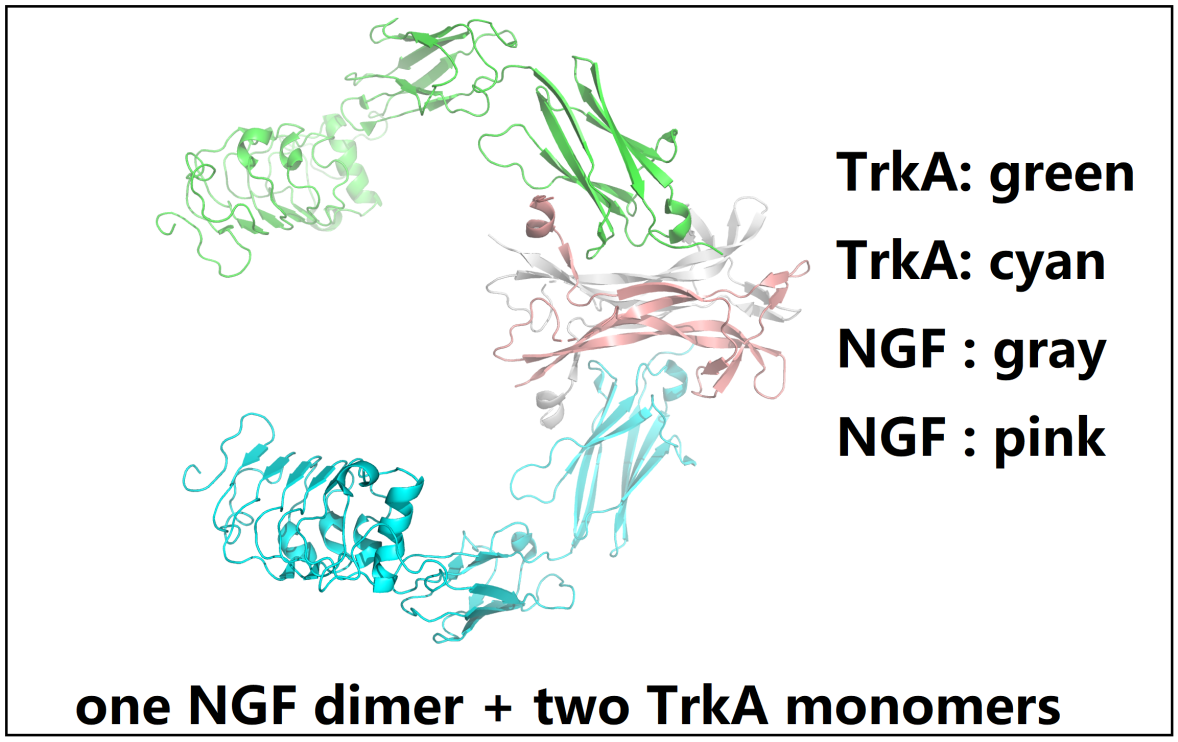

Figure 1. A overview of the experimental structure of the extracellular segment of human TRKA in complex with nerve growth factor (PDB ID **2IFG** [48]). This figure is prepared by PyMol [49].

Specifically, of the structure of the extracellular segment of human TRKA in complex with nerve growth factor with PDB ID **2IFG** [48], a set of relevant amino acid sequences are listed in italics in fasta format as below,

>TRKAchainA

CPDACCPHGSSGLRCTRDGALDSLHHLPGAENLTELYIENQQHLQHLELRDLRGLGELRNLTIV KSGLRFVAPDAFHFTPRLSRLNLSFNALESLSWKTVQGLSLQELVLSGNPLHCSCALRWLQRWEEEGL GGVPEQKLQCHGQGPLAHMPNASCGVPTLKVQVPNASVDVGDDVLLRCQVEGRGLEQAGWILTE LEQSATVMKSGGLPSLGLTLANVTSDLNRKNVTCWAENDVGRAEVSVQVNVSFPASVQLHTAVEM HHWCIPFSVDGQPAPSLRWLFNGSVLNETSFIFTEFLEPAANETVRHGCLRLNQPTHVNNGNYTLLA ANPFGQASASIMAAFMDNP

>TRKAchainB

CPDACCPHGSSGLRCTRDGALDSLHHLPGAENLTELYIENQQHLQHLELRDLRGLGELRNLTIV KSGLRFVAPDAFHFTPRLSRLNLSFNALESLSWKTVQGLSLQELVLSGNPLHCSCALRWLQRWEEEGL GGVPEQKLQCHGQGPLAHMPNASCGVPTLKVQVPNASVDVGDDVLLRCQVEGRGLEQAGWILTE LEQSATVMKSGGLPSLGLTLANVTSDLNRKNVTCWAENDVGRAEVSVQVNVSFPASVQLHTAVEM HHWCIPFSVDGQPAPSLRWLFNGSVLNETSFIFTEFLEPAANETVRHGCLRLNQPTHVNNGNYTLLA ANPFGQASASIMAAFMDNP

>NGFchainE

SSSHPIFHRGEFSVCDSVSVWVGDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCRDPNPV DSGCRGIDSKHWNSYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAVRRA

>NGFchainF

SSSHPIFHRGEFSVCDSVSVWVGDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCRDPNPV DSGCRGIDSKHWNSYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAVRRA

To further elucidate the mechanism of the design of NGF analogues to suppress pain signal transduction mediated by the p75NTR-NGF-TrkA complex here, Figure 2 shows a closer look of the homodimer of two native NGF molecules, as experimentally determined by X-ray diffraction in 2007 (PDB ID **2IFG** [48]), as the core of the idea is that NGF analogues are to be still able to bind two

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neurotrophin receptors: p75 neurotrophin receptor (p75NTR) and tyrosine kinase receptor A (TrkA), yet, their ability to form a homodimer (Figure 2) is inhibited via disruptions of a set of key inter-residue interactions [50] at the binding interface of native NGF homodimers (Figure 1).

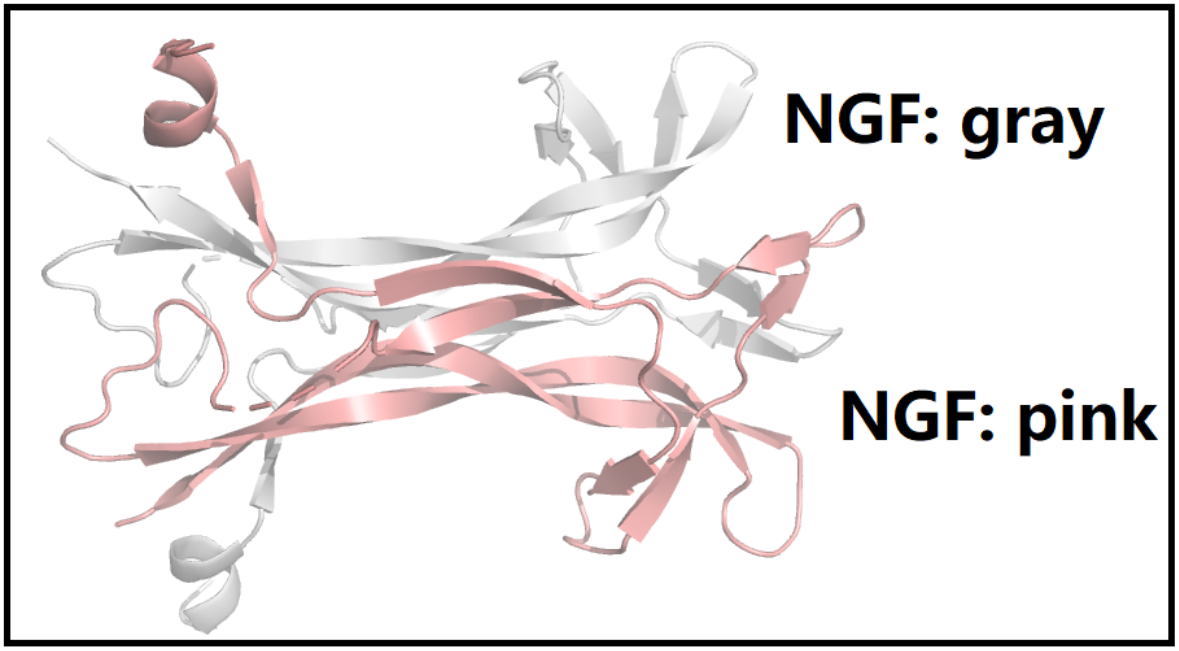

Figure 2. A closer look of the homodimer of two native NGF molecules as determined by X-ray diffraction (PDB ID **2IFG** [48]). This figure is prepared by PyMol [49].

2.1. A Comprehensive Structural and Biophysical Analysis of the p75NTR-NGF-TrkA Complex

As described previously in [50–52], a comprehensive structural and biophysical analysis was conducted for the structure of the extracellular segment of human TRKA in complex with nerve growth factor with PDB ID **2IFG** [48]. Specifically, the salt bridge analysis was conducted with an in-house python script only for titrateable residues (Asp, Glu, Lys, Arg and His), 4.0 Å was used as the cutoff distance for the two oppositely charged groups [50]. The hydrogen bond analysis was also conducted for only side chain nuclei with an in-house python script, and employed two geometric criteria: (a) a cutoff value of the angle formed by acceptor (A), donor (D) and hydrogen (H) ($\angle ADH$) of 30°; (b) a cutoff value of donor-acceptor distance at 3.0 Å. That is, a hydrogen bond is only considered to be formed if $\angle ADH$ is no larger than 30° and the donor-acceptor distance is not larger than 3.0 Å [50].

2.2. The Design of NGF Analogues to Suppress Pain Signal Transduction Mediated by the p75NTR-NGF-TrkA Complex

In combination with the comprehensive structural and biophysical analysis [50] as described above, the key amino acid residues at the p75NTR-NGF-TrkA complex binding interface (PDB ID: 2IFG) were examined carefully [**?**] in PyMol [49], and the inter-residue distances were calculated by PyMol [49] to identify potential neighbouring residue pair(s) to modulate the structural stability of the p75NTR-NGF-TrkA complex structure, leading to the design of a set of NGF analogues with reduced affinity such that ability of NGF monomers to form a homodimer (Figure 2) is inhibited via disruptions of a set of key inter-residue interactions [50] at the binding interface of native NGF homodimers (Figure 1). Specifically, after homology structural modeling with Modeller [53], the binding affinity between rt-PA analogue and PAI-1 was calculated using Prodigy [54,55].

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3. Results

As described above, the core of the idea is that NGF analogues are to be still able to bind two neurotrophin receptors: p75 neurotrophin receptor (p75NTR) and tyrosine kinase receptor A (TrkA), yet, their ability to form a homodimer (Figure 2) is inhibited via disruptions of a set of key inter-residue interactions [50] at the binding interface of native NGF homodimers (Figure 1). Essentially, the design of NGF analogues here is the construction of an n-dimensional NGF dimer-based mini GIBAC [52] towards the suppression of chronic NGF-related pain signaling, where n represents the number of site-specific mutations introduced to the amino acid sequence of NGF, as listed below:

>NGFchainE

SSSHPIFHRGEFSVCDSVSVWVGDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCRDPNPV DSGCRGIDSKHWNSYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAVRRA

>NGFchainF

SSSHPIFHRGEFSVCDSVSVWVGDKTTATDIKGKEVMVLGEVNINNSVFKQYFFETKCRDPNPV DSGCRGIDSKHWNSYCTTTHTFVKALTMDGKQAAWRFIRIDTACVCVLSRKAVRRA

Given that the length of monomeric NGF is 121, n is not to be beyond six to ensure that the NGF analogues is more than 95% homologous to its native counterpart, and the accuracy of the homology structural modeling using the experimental structure of the extracellular segment of human TRKA in complex with nerve growth factor (PDB ID **2IFG** [48]).

Here, in this article, a total of three occasions were reported, i.e., $n = 3$, specifically,

1. $n = 1$, i.e., a one-dimensional NGF dimer-based mini GIBAC [51,52] for the design of NGF analogues towards the suppression of NGF-related chronic pain signaling, as partly listed in Table 2.
2. $n = 2$, i.e., a two-dimensional NGF dimer-based mini GIBAC [51,52] for the design of NGF analogues towards the suppression of NGF-related chronic pain signaling, as partly listed in Table 3.
3. $n = 3$, i.e., a three-dimensional NGF dimer-based mini GIBAC [51,52] (Supplementary file **trio.pdf**) for the design of NGF analogues towards the suppression of NGF-related chronic pain signaling, as partly listed in Table 4.

Mutation	Energy mean	Energy std	K_d mean	K_d std
S4F	-10.270	0.671	8.953000000000001e-08	6.715793400634061e-08
S4A	-10.310	0.342	6.23e-08	3.199703111227665e-08
S4P	-10.320	0.389	6.8e-08	6.065311203887233e-08
S4V	-10.370	0.335	5.59e-08	2.6135990511170603e-08
S4I	-10.370	0.377	6.05e-08	4.102499238269277e-08
S4M	-10.450	0.478	5.9399999999999996e-08	4.9218289283558e-08
S4L	-10.560	0.578	5.859e-08	7.30497973987608e-08
S4Y	-10.650	0.301	3.52e-08	1.70926884953772e-08
S4G	-10.660	0.258	3.24e-08	1.3162066707018317e-08
S4C	-10.850	0.461	2.911e-08	1.89068479657504e-08
A98S	-10.860	0.595	3.832e-08	5.483358095182185e-08
S4K	-11.020	0.328	1.958e-08	1.4229111005259603e-08
A98Q	-11.070	0.454	2.023e-08	1.62038297942184e-08
A98T	-11.150	0.364	1.6529999999999996e-08	1.0195494102788742e-08
S4E	-11.180	0.464	1.726e-08	1.4366293885341482e-08
S104I	-11.180	0.447	1.645e-08	1.0609736094738644e-08
S4D	-11.200	0.410	1.5650000000000004e-08	1.0420772524146184e-08
S104L	-11.260	0.250	1.24e-08	3.7812696280482305e-09
N36A	-11.260	0.242	1.24e-08	4.991192242340502e-09

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S4W	-11.280	0.440	1.394e-08	8.486247698482527e-09
N36P	-11.310	0.262	1.1369999999999999e-08	4.242652472215937e-09
I35S	-11.370	0.618	1.4880000000000002e-08	1.4351431984300383e-08
S104P	-11.420	0.392	1.025e-08	5.6498230060772695e-09
S104F	-11.420	0.421	1.1350000000000002e-08	8.690368231553827e-09
S104G	-11.430	0.473	1.126e-08	7.755282070950095e-09
S4H	-11.440	0.472	1.229e-08	1.466243158551814e-08
S104A	-11.460	0.361	9.48e-09	4.582313826005372e-09
N36G	-11.470	0.323	9.34e-09	5.523078851510269e-09
I35T	-11.500	0.490	1.1449999999999998e-08	1.2621509418449125e-08
S104V	-11.540	0.508	1.091e-08	1.3590912404985913e-08
Nddm	-12.700	0.000	1.2e-09	0.0

Table 2. Single site-specific mutation-based analysis of the binding affinities between two nerve growth factor monomers. Prodigy [55] is used to calculate the binding energy and K_d between two nerve growth factor monomers. In this table, the row for **Nddm** (yellow background) represents the binding affinity between two native nerve growth factor monomers.

Mutation	Energy mean	Energy std	K_d mean	K_d std
S4F_A98G	-8.600	0.000	9.1e-07	0.0
S4A_L81D	-8.600	0.000	8.3e-07	0.0
S4Y_L81A	-8.700	0.000	7.8e-07	0.0
S4A_I22G	-8.800	0.000	6.3e-07	0.0
S4A_D63G	-8.800	0.000	6e-07	0.0
S4G_A98Q	-8.900	0.000	5.5e-07	0.0
S4G_A88E	-8.900	0.000	5.2e-07	0.0
S4A_V33A	-8.900	0.000	5.1e-07	0.0
S4F_D63I	-9.000	0.000	4.6e-07	0.0
S4A_A98Q	-9.000	0.000	4.4e-07	0.0
S4Y_G1Q	-9.100	0.000	3.8e-07	0.0
S4A_I22F	-9.100	0.000	3.9e-07	0.0
S4G_E46D	-9.100	0.000	4.1e-07	0.0
S4G_K79E	-9.100	0.000	3.7e-07	0.0
S4G_H66A	-9.100	0.000	3.9e-07	0.0
S4A_K106L	-9.100	0.000	3.9e-07	0.0
S4Y_A89L	-9.200	0.000	3.4e-07	0.0
S4G_W90P	-9.200	0.000	3.4e-07	0.0
S4A_K65R	-9.200	0.000	3.2e-07	0.0
S4G_L81E	-9.300	0.000	2.6e-07	0.0
S4A_I95A	-9.300	0.000	2.9e-07	0.0
S4G_L30M	-9.300	0.000	2.9e-07	0.0
S4G_W90G	-9.300	0.000	2.7e-07	0.0
S4L_H75I	-9.300	0.000	3e-07	0.0
Nddm	-12.700	0.000	1.2e-09	0.0

Table 3. Double site-specific mutation-based analysis of the binding affinities between two nerve growth factor monomers. Prodigy [55] is used to calculate the binding energy and K_d between two nerve growth factor monomers. In this table, the row for **Nddm** (yellow background) represents the binding affinity between two native nerve growth factor monomers.

As quantitatively described in Tables 2, 3 and 4, the binding affinity (K_d) between two native nerve growth factor monomers at 37 °C is 1.2 $\times$ 10^{-9} M, while the binding affinity (K_d) between two nerve growth factor analogue monomers at 37 °C is reduced to as low as 5.8 $\times$ 10^{-6} M (i.e., the

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S4A_A98Q_V33A site-specific mutations for native NGF monomers) with a set of triple site-specific mutations introduced into the amino acid sequence of native NGF sequence, towards the disruption of the homodimer of NGF and the suppression of NGF-related chronic pain signaling mediated by the p75NTR-NGF-TrkA complex (PDB ID **2IFG** [48]).

Mutation	Energy mean	Energy std	K_d mean	K_d std
S4A_A98Q_V33A	-7.400	0.000	5.8e-06	0.0
S4A_A98Q_V33G	-7.400	0.000	6.1e-06	0.0
S4G_A98Q_K79P	-7.500	0.000	4.7e-06	0.0
S4G_A98Q_W90A	-7.500	0.000	4.8e-06	0.0
S4Y_A98Q_R105I	-7.500	0.000	5.5e-06	0.0
S4Y_A98Q_K41P	-7.600	0.000	4.2e-06	0.0
S4F_A98Q_Y70L	-7.700	0.000	3.5e-06	0.0
S4G_A98Q_W90V	-7.700	0.000	4e-06	0.0
S4F_A98Q_K41V	-7.700	0.000	3.5e-06	0.0
S4G_A98Q_K79M	-7.700	0.000	3.5e-06	0.0
S4A_A98Q_L81A	-7.700	0.000	3.5e-06	0.0
S4Y_A98Q_K41V	-7.800	0.000	3.2e-06	0.0
S4A_A98Q_W12G	-7.800	0.000	3.1e-06	0.0
S4F_A98Q_L81K	-7.800	0.000	3e-06	0.0
S4G_A98Q_Q42G	-7.800	0.000	2.9e-06	0.0
S4L_L81G_I35Q	-7.800	0.000	3e-06	0.0
S4G_A98Q_K41P	-7.800	0.000	3.4e-06	0.0
S4G_A98Q_D63G	-7.900	0.000	2.9e-06	0.0
S4F_A98Q_K79G	-7.900	0.000	2.5e-06	0.0
S4G_A98Q_R105G	-7.900	0.000	2.5e-06	0.0
Nddm	-12.700	0.000	1.2e-09	0.0

Table 4. Triple site-specific mutation-based analysis of the binding affinities between two nerve growth factor monomers. Prodigy [55] is used to calculate the binding energy and K_d between two nerve growth factor monomers. In this table, the row for **Nddm** (yellow background) represents the binding affinity between two native nerve growth factor monomers.

4. Conclusions and Discussion

Overall, this article for the first time puts forward a set of NGF analogues, including in particular NGF analogues with four site-specific mutations, for whose dimerizations the K_d at 37 °C were reduced by three orders of magnitude (from 10^{-9} M to 10^{-6} M) compared to the K_d at 37 °C for the dimerization of native NGFs. As discussed above, a structural and biophysical perspective (Figure 3) is crucial in guiding the rational design of these analogues, ensuring a nuanced understanding of the intricate molecular mechanisms involved in pain transduction and enabling the development of therapeutics with enhanced specificity and efficacy [56–60]. Hence, the investigation here of the design of NGF analogues, targeted at suppressing pain signal transduction mediated by the p75 neurotrophin receptor (p75NTR)-NGF-tyrosine kinase receptor A (TrkA) complex, has yielded valuable insights into the potential for developing innovative analgesic interventions [61–65].

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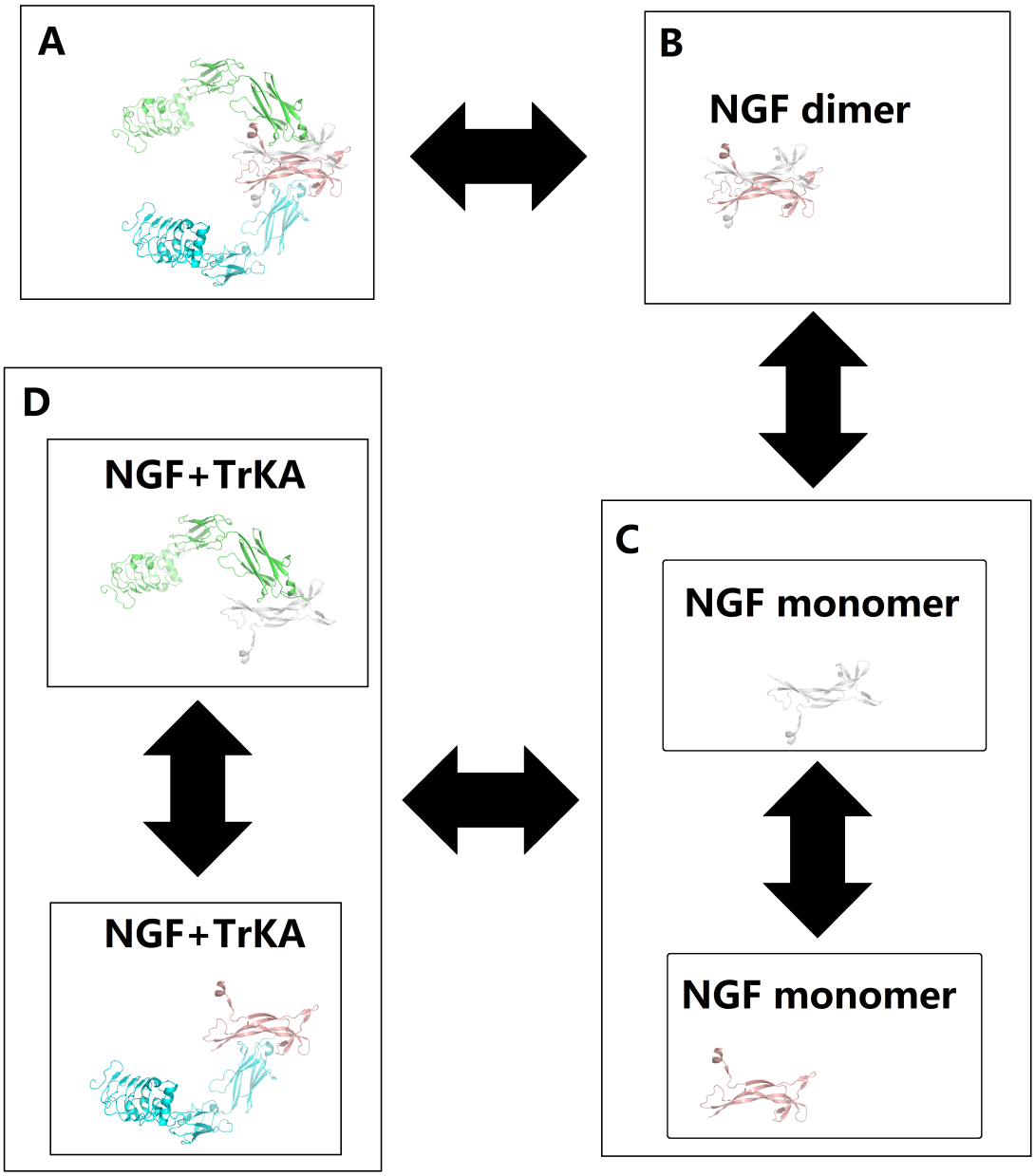

Figure 3. Flowchart of the design of NGF analogues to disrupt the NGF homodimer and suppress pain signal transduction mediated by the p75NTR-NGF-TrkA complex.

Of further pharmaceutical interest is the integration of experimental biophysical techniques complemented our computational predictions, offering a dynamic perspective on the interactions between the designed analogues and the complex [66–69]. In the realm of drug discovery and design, the quest for innovative therapeutic interventions to alleviate chronic pain has become a pivotal focus. Chronic pain, a pervasive and debilitating condition, poses a significant clinical challenge, necessitating the exploration of novel targets and strategies. Among the various molecular players implicated in pain signal transduction, the p75 neurotrophin receptor (p75NTR)-nerve growth factor (NGF)-tyrosine kinase receptor A (TrkA) complex has emerged as a promising nexus for intervention [70–74].

In short, the use of NGF analogues in drug discovery heralds a paradigm shift in the approach to pain modulation. By harnessing insights from structural and biophysical perspectives, researchers can tailor NGF analogues to disrupt specific interactions within the p75NTR-NGF-TrkA complex. This

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precision allows for the design of therapeutics with the potential to selectively dampen pain signaling pathways while minimizing off-target effects [75–82].

In conclusion, the structural and biophysical perspective on designing NGF analogues to suppress pain signal transduction provides a robust foundation for the development of targeted analgesic therapies. By elucidating the structural biophysics underlying the p75NTR-NGF-TrkA complex, this article contributes valuable knowledge to the field, paving the way for the next generation of therapeutic strategies aimed at alleviating chronic pain [83–91].

5. Ethical statement

No ethical approval is required.

6. Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author used OpenAI's ChatGPT in order to improve the readability of the manuscript, and to make it as concise and short as possible. After using this tool, the author reviewed and edited the content as needed and takes full responsibility for the content of the publication.

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