Molecular basis of lateral force spectroscopy nano-diagnostics: computational unbinding of autism related chemokine MCP-1 from IgG antibody

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Abstract Monocyte-chemoattractant protein-1 (MCP-1), also known as CCL2, is a potent chemoattractant of T cells and monocytes, involved in inflammatory and angio-proliferative brain and retinal diseases. Higher expression of MCP-1 is observed in metastatic tumors. Unusual levels of MCP-1 in the brain may be correlated with autism. Immunochemistry where atomic force microscope (AFM) tips functionalized with appropriate antibodies against MCP-1 are used could in principle support medical diagnostics. Useful signals from single molecule experiments may be generated if interaction forces are large enough. The chemokine-antibody unbinding force depends on a relative motion of the interacting fragments of the complex. In this paper the stability of the medically important MCP-1- immunoglobulin G antibody Fab fragment complex has been studied using steered molecular dynamics (SMD) computer simulations with the aim to model possible arrangements of nano-diagnostics experiments. Using SMD we confirm that molecular recognition in MCP1-IgG is based mainly on six pairs of residues: Glu39A - Arg98H, Lys56A - Asp52H, Asp65A - Arg32L, Asp68A - Arg32L, Thr32A - Glu55L, Gln61A - Tyr33H. The minimum external force required for mechanical dissociation of the complex depends on a direction of the force. The pulling of the MCP-1 antigen in the directions parallel to the antigen-antibody contact plane requires forces about 20 %-40 % lower than in the perpendicular one. Fortunately, these values are large enough that the fast lateral force spectroscopy may be used for effective nano-diagnoses purposes. We show that molecular modeling is a useful tool in planning AFM force spectroscopy experiments.

Keywords Atomic force microscopy · Bionanomechanics · IgG antibody · Monocyte-chemoattractant protein-1 · Steered molecular dynamics

Introduction

The immune system (IS) is a complex network of body organs providing means for defense against pathogens and maintaining integrity of the organism [1]. Due to its medical significance, especially in the contexts of allergies and cancer immunotherapy, IS is vigorously studied [2]. However, our understanding of IS, especially on a molecular level, is still not satisfactory. Recent years brought discoveries of new roles of signaling proteins involved in IS activity modulating the brain [3]. Here we investigate interactions of a small protein MCP-1 which has a diagnostic potential in diseases related to the central nervous system (CNS) and cancer.

Monocyte chemoattractant protein-1 (MCP-1), also known as chemokine ligand 2 (CCL2), is a member of the chemokine family [4]. The chemokines are a type of cytokines, they are divided into two main groups, based on a relative position of the conserved cysteines, which can be either adjacent (CC type) or separated by one amino acid (CXC chemokines). The basic function of those cytokines is to act on chemoattraction in traffic regulation of immune cells. MCP-1 consists of 77 amino acids and belongs to the CC subfamily [5]. It binds to CCR2 and CCR4 receptors [6] but interacts strongly with specialized antibodies as well. MCP-1 is a chemoattractor to monocytes, memory T cells, and dendritic cells [7, 8]. Its main function is to recruit those immune cells to a place of infection or injury. Over a thousand of experimental papers on MCP-1 have been published since its discovery in 1989 [9, 10].
Recent studies show that MCP-1 is also expressed in astrocytes, microglia and neurons [11, 12]. Receptors of MCP-1 are present on surfaces of those CNS cells. The presence of MCP-1 causes migration of leukocytes into the CNS. MCP-1 is present in CNS not only in inflammation but also in the healthy brain [4]. Thus, this chemokine can modulate activity of neurons, astrocytes and microglia [3].

MCP-1 may play a role in many diseases, including multiple sclerosis, rheumatoid arthritis, atherosclerosis, obesity and insulin-resistant diabetes [13]. It has a direct role in angiogenesis and tumor progression [14], promotes prostate cancer tumorigenesis and metastasis [15]. The first studies on humans of a new drug CNTO888 (monoclonal antibody) blocking MCP-1 has been very recently published [16]. Other recent studies show that it may also be involved in autism spectrum disorder (ASD) [17–19]. The level of MCP-1 in brain tissues and cerebrospinal fluid [17] in autistic subjects is higher than that in healthy people. Levels of MCP-1 in plasma of children with ASD is elevated [18] as well. Those facts may suggest that the neuroimmune response is a part of the neuropathological processes in ASD and that MCP-1 may play a pathogenic role in this disorder [19] and prompted us to undertake this study.

Atomic force microscopy (AFM) has been proposed as a useful tool for studies of molecular recognition processes [20, 21]. Protein-antibody interactions may be studied using classical contact mode techniques [22–24]. In these methods the antibody is attached to a silanized tip and a vertical force exerted by the AFM cantilever tip dissociates transient antibody-protein complexes [25, 26]. The maximum force registered during withdrawing the cantilever from the surface covered with a sample, is a measure of the interaction between antibody and protein. The AFM techniques have been successfully used in other antigen-IgG studies [27, 28] but these are very time-consuming procedures. The MCP-1 IgG complex has not been studied experimentally, yet. An alternative, promising in the nano-diagnostics, technique is friction (or lateral) force spectroscopy (FFS) [29, 30]. In contrast to the standard contact mode AFM, in FFS the probe quickly scans the surface laterally and the “unbinding” is enforced by the lateral forces. Usually the AFM tip is functionalized by the antibody and a protein is immobilized on a surface. The process of the enforced dissociation (molecular recognition) may depend on a relative orientation/motion of both proteins. Forces too weak do not generate useful signals.

In order to compare intermolecular interactions met in a classical AFM recognition experiment and in the lateral arrangement, we set-up a computer model of such an experiment. The impact of the direction of the AFM tip motion on mechanically enforced dissociation of antigen—antibody complex was computationally studied. Simulations of MCP-1 in a complex with the Fab fragment of immunoglobulin IgG antibody were performed using the Steered Molecular Dynamics method (SMD) [31–33]. This “virtual experiment” approach has been successful in studies of proteins’ nanomechanics [34–36]. Our numerous SMD ns scale trajectories revealed new atomic details of the molecular recognition phenomena in that medically important system. We show that the lateral dissociation requires substantially (20–40 %) lower forces than the vertical uncoupling of the complex but the later forces are still high enough to make FFS in our system feasible. This finding indicates that after careful calibration of friction based AFM methods a routine FFS nano-diagnostics involving MCP-1 chemokine will be possible.

Methods

As the first step we studied dynamics of a complex of human MCP-1 with 11 K2 Fab fragment [6] (Fig. 1) using the molecular dynamic (MD) method [37]. 11 K2 is a mouse monoclonal antibody against several human MCPs. The complex, extracted from the 2BDN entry in the Protein Data Bank was embedded in an 8 Å thick TIP3P water shell. After 0.4 ns equilibration we performed 10 ns of main MD simulation, using the NAMD 2.7 code [38] and the CHARMM27 force field [39].

Next, the SMD [31, 32] method was used in order to apply an external force which should dissociate the MCP-1-antibody complex in two perpendicular directions: the „vertical” force (V, almost parallel to the main axis of the antibody, the direction „z” in Fig. 1) and the „lateral” one (L, approximately perpendicular to the main axis of the antibody). An increasing external virtual force was attached to all CA atoms of MCP-1 (chain A). During the simulations of stretching all CA atoms of

![Fig. 1](image-url) A schematic view of MCP-1 (blue) and Fab fragment of Ig G antibody complex. In red light chains (L) are shown, in gray heavy chains (A) are depicted. V denotes variable region of IgG. Yellow arrows represent directions of virtual forces used in simulations. An orange arrow is an example of V direction and a green one represents L direction. Note that direction of the z axis was used to define spherical coordinates of force vectors.
the antibody (chains L and H) were fixed. The last structures obtained from the 3 ns standard MD simulations served as starting points for all SMD simulations. Structures were pulled for 2 ns at a constant speed of 0.025 Å/ps with a spring constant of 278 pN/Å. This value is close to that used in typical FFS experiments. Twenty two pulling directions were used. In addition we have studied a role of disulfide bridges on this molecular recognition process: two 2 ns simulations for each direction were generated for systems with all disulfide bridges converted to cysteines. Thus 3×9 V trajectories, and 3×13 L trajectories (Fig. 1) were further analyzed. Moreover, for one selected, representative V direction and one L direction ten additional trajectories (2 ns each) were generated in order to calculate values of an average dissociation force and to estimate statistical errors in the maximum force determination. Additionally, a dependence of the calculated forces on the pulling speed was tested. For ten directions, five vertical (V) and five lateral ones (L), we generated trajectories with a constant speed of 0.0025 Å/ps, i.e., ten times slower than before.

Electrostatic molecular potentials were calculated using the APBS method [40–43].

The analysis of results was performed using the VMD code [44] and homemade scripts.

Results and discussion

A classical MD

Since MCP-1 chemokine, despite its medical significance, has not been previously analyzed using classical MD modeling, we have studied dynamics of the complex on a 10 ns timescale. Except for the flexible terminal ends the chemokine has a rigid structure. Mean square atomic displacements of amino acids with respect to average positions (B-factor simulation) correlate rather well with the temperature B-factors (Fig. 2).

As expected, the N-terminal end (Ala4-Thr10) exhibit very large flexibility. This region is responsible for dimerization of MCP-1 cytokine [45]. One may notice that in the Cys12–Ile32 fragment the model is more stable than X-ray measurements indicate. Probably in the computer model of an isolated complex the Arg18 residue is more strongly stabilized by the intramolecular electrostatic interactions than in a crystal setting. Both the simulations and the X-ray experiment indicate that the most stable region is Ala40–Thr45 which corresponds to β2 internal β stand. Amino acids identified in the SMD simulations as being involved in molecular recognition process (Thr32, Glu39, Lys56, Gln61, Asp65) exhibit low fluctuations as well. This means that the MCP-1/IgG interface is quite well stabilized. The calculated B-factors for the Fab fragment are also in a very good agreement with the experimental data [6], Fig. 3.

In Fig. 3 one can see that the most flexible part of the antibody heavy chain corresponds to a large loop in the region from Ala128(H) to Ser138(H). Interestingly, also in the antibody we observe a polar region which is more stable in the simulations than in a real crystal: Glu175-Ser176-Asp177. We explain this stabilization by a relaxation in an isolated model complex which leads to new salt bridges. Probably such relaxation is absent in the crystal due to packing interactions.

Steered molecular dynamics—mechanically enforced dissociation

All calculated force spectra of the enforced dissociation of MCP1-Fab complexes show qualitatively the same features—(i) a steep rise of the force up to a certain maximum value, (ii) a gradual decrease of the interaction force (iii) a separation phase characterized by a force close to 1 nN corresponding to the hydrodynamic drag. Representative curves for L and V dissociation modes are shown in Fig. 4. All calculated maxima of unbinding forces are collected in Table 1.

![Fig. 2](image1.png)  
**Fig. 2** A comparison of calculated mean square atomic displacements of MCP-1 cytokine amino acids with experimental temperature B-factors [6]

![Fig. 3](image2.png)  
**Fig. 3** A comparison of calculated mean square atomic displacements of heavy chain of Fab IgG antibody fragment with experimental temperature B-factors [6]
In reducing chemical conditions S-S protein bridges may break apart, dithiothreitol is commonly used for that purpose. Also some enzymes, such as thioredoxin or glutaredoxin facilitate transfer of electrons and make this redox reaction possible. Another source of the absence of stabilizing S-S covalent bonds may be just a point mutation in a cysteine position. Such perturbation may have significant impact on the molecular recognition process, particularly in CC chemokines. In order to check how such a structural modification may affect “in vitro” AFM experiments, we have repeated SMD simulation for models in which S-S bridges were disrupted both in MCP-1 chemokine and in the IgG antibody Fab fragment (7 bridges in total are absent now). Results for the reduced forms of the complex are presented in Table 1 as well (First, Second).

Thus, in total 69 output files were analyzed for the value of the force necessary to dissociate the complex.

### Table 1

| Direction vector V | SS_present | First  | Second | L      | SS_present | First  | Second |
|--------------------|------------|--------|--------|--------|------------|--------|--------|
| V1                 | 2102       | 1791   | 2213   | L11    | 1522       | 1856   | 2001   |
| V2                 | 2911       | 2342   | 1943   | L12    | 2045       | 1958   | 1497   |
| V3                 | 2678       | 1989   | 2448   | L13    | 1668       | 1622   | 1877   |
| V4                 | 2663       | 2195   | 2215   | L14    | 1391       | 1551   | 1511   |
| V5                 | 2084       | 2264   | 2294   | L15    | 1859       | 1760   | 1548   |
| V6                 | 1768       | 1961   | 1671   | L16    | 1737       | 2330   | 2293   |
| V7                 | 2537       | 2418   | 2208   | L17    | 2148       | 1859   | 2001   |
| V8                 | 2152       | 2293   | 2239   | L18    | 1988       | 2170   | 1959   |
| V10                | 2776       | 2611   | 2431   | L19    | 2229       | 2003   | 2033   |
| V5v2               | 2161       | 2423   | 2323   | L20    | 1606       | 1798   | 1604   |
| V5v3               | 2214       | 2061   | 2294   | L21    | 1472       | 1608   | 1556   |
| V5v4               | 1907       | 2067   | 2178   | L22    | 2224       | 2076   | 2127   |
| V5v5               | 1974       | 2345   | 2259   | L23    | 1493       | 1856   | 2255   |
| Average            | 2302       | 2212   | 2209   | Average| 1799       | 1880   | 1866   |
| Standard deviation | 366        | 228    | 204    | Standard deviation | 301 | 227 | 289 |
| V2 slower10x       | 1591       | L14 slower10x | 905 |
| V7 slower10x       | 1259       | L20 slower10x | 829 |
| V10 slower10x      | 1515       | L21 slower10x | 793 |
| V5 slower10x       | 1097       | L13 slower10x | 1159 |
| V8 slower10x       | 1723       | L15 slower10x | 1124 |
| Average            | 1437       | Average| 962    | Standard deviation | 169 |
| Standard deviation | 254        | Standard deviation | 169 |
In the standard forms (S-S bonds present), for 2 ns simulations, when the vertical (V) direction of the pulling force vector was applied, the lowest calculated force value was 1768 pN, while the highest value was 2911 pN. The average value of the maximum force observed during V direction SMD simulation was 2302±366 pN. For laterally oriented pulling vectors the forces were lower: the highest value was 2229 pN, the lowest was 1391 pN, the average 1799±301 pN. Respectively, for the simulations with ten times slower pulling speed the average for the vertical forces was 1437±254 pN, and for the lateral force simulations the average was 962±169 pN. Thus the process of mechanical unbinding requires lower forces if it proceeds in the direction L parallel to the MCP-1-antibody contact plane.

To study possible correlations between the value of the force necessary to separate antibody and MPC-1 and the dragging force direction we transformed force vectors into standard spherical coordinates (see Fig. 1). Forces with respect to values of the φ angle (only for "lateral" L cases) are given in Fig. 5a and forces dependence on the θ angle are shown in Fig. 5b.

Data presented in Fig. 5 clearly show that for the purely vertical separations the process requires the highest forces. In more lateral directions some lower force paths for dissociation may be easily found. The average later force is 22 % (2 ns simulations) and 40 % (for ten times slower pulling speed) lower than the vertical one. One should remember, that the SMD calculated forces, even with the low pulling speed, are a factor of 8–10 higher than those usually measured in AFM FS experiments. The reason is that, due to limited computer power, the loading rate used in SMD calculations has to be typically much higher than that in an experiment. However, calculated SMD forces correctly reproduce experimental trends: the lower the pulling speed—the lower the force. The simulations with ten times lower velocities confirm, that there is a difference in maximum unbinding forces for V and L arrangements of the dissociating components of the complex. Given all difficulties related to quantitative reproduction of force spectra from the
virtual SMD experiments, we conclude that lateral motion of the functionalized AFM tip may require less force than a standard (V) contact mode molecular recognition study. These findings await for experimental confirmation, so far no AFM experiments for this system have been performed yet.

Molecular recognition and bioinformatics analysis of MCP-1

Detailed analysis of trajectories gave unique information on molecular interactions between the antibody and MCP-1 and their evolutions along alternative stretching paths. The flat interface region in a static crystal has been previously characterized [6]: it consists of the end of $\beta_{1}$ strand, the $\beta_{1}$–$\beta_{2}$ loop, the beginning of $\beta_{2}$ strand, the loop between $\beta_{3}$ and $\alpha_{1}$, and the $\alpha_{1}$ helix. The formation of the complex buries about 15 % of the MCP-1 solvent accessible surface. Most of the contacts with MCP-1 are made through complementarity determining regions (CDR) H1 and H3 of the heavy chain of IgG and CDRs L1 and L3 of the light chain [6]. Important for recognition is Phe101H residue embedded in a mainly hydrophobic pocket of MCP-1 composed of Arg30A, Thr32A, Glu39A, Val41A, Pro55A and Met64A.

During stretching we observe that the complex is stabilized by strong salt bridges (in bold) and hydrogen bonds between: Glu39A - Arg98H, Lys56A - Asp52H, Asp65A - Arg32L, Asp68A - Arg32L, Thr32A - Glu55L, Gln61A - Tyr33H, where A denotes MCP-1, L - a light chain of IgG Fab fragment and H—the heavy chain of Fab. These interactions are shown in Fig. 6. Enlisted salt bridges/H-bonds were present in the majority of analyzed trajectories prior to an enforced dissociation both in V and L subsets. However, the scenario of consecutive breaking of these bonds obviously depends on the direction of the dragging force, particularly for L type vectors. Our trajectories provide data for interpretation of fine details induced by local events in future AFM force spectra.

Reduction of cysteines does not affect this list of stronger intermolecular interactions in MCP-1/Fab complex. Interestingly, additional H-bonds interacting pairs Asp68A-Ile31L, Lys56A-Trp32H were observed only for the complex with S-S bonds present.

Besides perfect matching of VdW surfaces and a strong hydrophobic handle created by Phe101, electrostatic interactions may also play a significant role for long range recognition in this system. We have used APBS program [40–43] to calculate a map of the molecular electrostatic potential (MEP) of MCP-1 and the Fab fragment. Rigid separated structures extracted from the 2BDN data were used for calculations. Results are presented in Fig. 7.

There are at least three regions (a, b, c, Fig. 7) with higher values of MEP. The regions in MCP-1 have corresponding counterparts in the Fab system of opposite charge. Thus electrostatics contribute to the stability of this complex as well. These calculations help to identify regions crucial for effective recognition of the important MCP-1 chemokine.

The fragment of MCP-1 specifically recognized by an antibody is called epitope. In order to check to what extent amino acids present at the interface in our cytokine are conserved in other proteins we performed PSI-BLAST search in standard non-redundant protein sequences database and used ClustalW2 code [46, 47] to make alignments of ten most similar sequences. Results visualized with Jalview 2.7 program [48, 49] are presented in Fig. 8.

For proteins in this set over 80 % similarity to MCP-1 is observed. Amino acids important for strong interactions identified by SMD simulations were analyzed in greater detail. Two distinct groups of polar epitope amino acids are present in MCP-1: conserved set (Thr32, Glu39, Gln61, Asp68) and a specific set: Lys56 and Asp65. In the conserved set the same amino acids are present in nearly all similar proteins. Residues from the specific set are characteristic only for MCP-1 chemokine. This finding corresponds well with the observed 160-fold decrease of IgG antibody affinity to a Lys56Asn MCP-1 mutant described in [6]. Thus Lys56 and Asp65 are particularly strongly involved in specific recognition of this chemokine by our IgG antibody. It is worth noting that this region is distinct from that responsible for an interaction of MCP-1 with its natural membrane receptor. Mutational studies have
shown, that only a small subset of surface residues of human MCP-1 is important for effective interaction with CCR2 receptor: Tyr13, Arg24, Lys38, Lys49 [50]. None of these residues is involved in interaction with Fab fragment of IgG antibody studied here.

Conclusions

The presented computer modeling study was intended to check whether the lateral FFS AFM approach using antibody functionalized tips can be used in antigen-antibody molecular recognition studies and nano-diagnostics involving chemokines. Our results of over forty 2 ns and ten 10 ns long SMD simulations have shown that the interaction force between the Fab fragment of IgG antibody and MCP-1 is significant in lateral unbinding, thus such measurements should be possible. Computer experiments indicate that irrespective of a particular direction of the lateral unbinding forces, their values are systematically lower by about 20-40 % than the vertical ones. Ten times lower pulling speed data confirm qualitatively this conclusion. The lower forces require better sensitivity in the experiments than a standard, time consuming tapping modes, if FFS technique is to be used in the nano-diagnostics. This may be achieved by careful selection of high affinity antibodies against MCP-1 or a better design of the AFM hardware. The SMD force required to dissociate the complex studied here was even bigger than the force required to unfold the whole contactin 4 protein calculated with the same SMD protocol [51].

The strong intermolecular interactions arise from a set of 4-6 strong salt bridges and hydrogen bonds. MEP maps based of the CHARMM force field for MCP-1 and the antibody show high degree of electrical complementarity. Substantial hydrophobic interactions of Phe101 IgG residue, also noticed earlier [6], contribute to the molecular recognition process as well. Recent synthesis of human MCP-1 using a combination of solid phase peptide synthesis and native chemical ligation further enhances potential for medical applications of this interesting protein [52].

In summary, our modeling results indicate that the AFM FFS diagnostics using antibody functionalized tips for fast lateral scanning of a sample with immobilized cytokine may be a promising alternative to time consuming classical AFM measurements.

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References

1. Paul WE (2008) Fundamental immunology. Kluwer/Lippincott Williams & Wilkins, Philadelphia
2. Weiner LM, Surana R, Wang S (2010) Monoclonal antibodies: versatile platforms for cancer immunotherapy. Nat Rev Immunol 10(5):317–327
3. Garay PA, McAllister AK (2010) Novel roles for immune molecules in neural development: implications for neurodevelopmental disorders. Front Systic Neurosci 2:136. doi:10.3389/fnsys.2010.00136
4. Comerford I, McColl SR (2011) Mini-review series: focus on chemokines. Immunol Cell Biol 89(2):183–184
5. Deshmukh SL, Kremslev S, Amini S, Sawaya BE (2009) Monocyte chemotactrant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 29(6):313–326
6. Reid C, Rushe M et al (2006) Structure activity relationships of monocyte chemotactrant proteins in complex with a blocking antibody. Protein Eng Des Sel 19(7):317–324
7. Carr MW, Roth SJ, Luther E, Rose SS, Springer TA (1994) Monocyte chemotactrant protein 1 acts as a T-lymphocyte chemotactrant. Proc Natl Acad Sci U S A 91(9):3652–3656
8. Xu L, Warren M, Rose W, Wang J (1996) Human recombinant monocyte chemotactic protein and other CC chemokines bind and induce directional migration of dendritic cells in vitro. J Leukoc Biol 60(3):365–371
9. Yoshimura T, Robinson EA, Tanaka S, Appella E, Kuratsu J-I, Leonard EJ (1989) Purification and amino acid analysis of two human glioma-derived monocyte chemotactrantants. J Exp Med 169(4):1449–1459
10. Van Coillie E, Van Damme J, Opdenakker G (1999) The MCP/ eotaxin subfamily of CC chemokines. Cytokine Growth Factor Rev 10(1):61–86
11. Banisadr G, Gosselin RD, Mechighel P, Kitabgi P, Rostne W, Parsadaniantz SM (2005) Highly regionalized neuronal expression of monocyte chemotactrant protein 1 (MCP 1/CCL2) in rat brain: evidence for its colocalization with neurotransmitters and neuropeptides. J Comp Neurol 489(3):275–292
12. De Haas A, Van Weering HRJ, De Jong E, Boddeke HWGM, Biber KPH (2007) Neuronal chemokines: versatile messengers in central nervous system cell interaction. Mol Neurobiol 36(2):137–151
13. Panee J (2012) Monocyte chemotactrant protein 1 (MCP-1) in obesity and diabetes. Cytokine 60(1):1–12
14. Salcedo R, Ponce ML et al (2000) Human endothelial cells express CCR2 and respond to MCP-1: direct role of MCP-1 in angiogenesis and tumor progression. Blood 96(1):34–40
15. Zhang J, Patel L, Pianta KJ (2010) CC chemokine ligand 2 (CCL2) promotes prostate cancer tumorigenesis and metastasis. Cytokine Growth Factor Rev 21(1):41–48
16. Sandhu SK, Papadopoulos K et al (2013) A first-in-human, first-in-class, phase I study of carlumab (CNTO 888), a human monoclonal antibody against CC-chemokine ligand 2 in patients with solid tumors. Cancer Chemother Pharmacol 71(4):1041–1050
17. Vargas D, Nascimbene C, Krishnan C, Zimmerman A, Pardo C (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57(1):67–81
18. Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah IN, Van de Water J (2011) Associations of impaired behaviors with elevated plasma chemokines in autism spectrum disorders. J Neuroimmunol 232(1–2):196–199
19. Pardo CA, Eberhart CG (2007) The neurobiology of autism. Brain Pathol 17(4):434–447
20. Parot P, Dufrenne YF, Hinterdorfer P, Le Grimellec C, Navajas D, Pellequer JL, Scheuring S (2007) Past, present and future of atomic force microscopy in life sciences and medicine. J Mol Recognit 20(6):418–431
