Chapter 1

Supramolecular Systems as Protein Ligands

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Abstract The standard substrate complexation mechanism engages natural binding sites. In contrast, supramolecular structures may form complexes with proteins by penetrating in regions which are either naturally unstable or become temporarily accessible due to structural rearrangements related to the protein's function. This may result in enhancement of irreversible processes (e.g. immune complexation or complement activation) or inhibition of reversible processes (e.g. enzymatic catalysis). Only ribbon-like supramolecular structures may form complexes with proteins. Having anchored itself inside the protein, the supramolecular ligand is protected against environmental factors such as changes in pH. This type of interaction represents a unique, nonstandard phenomenon in the context of proteomics.

Keywords Protein dynamics and Congo red binding • Ribbon-like supramolecular micelles • Congo red as supramolecular dye • Self-assembled molecules form a unit protein ligand • Unity of self-assembled molecules • Congo red penetration to protein interior • Congo red complexation properties • Protection of bound Congo red by proteins

1.1 Mechanism of Complexation

Biological function is a critical aspect in proteomics, and is often defined as the capability to interact with specific ligands and form complexes. Protein ligands tend to be either small molecules or small fragments of larger systems. They bind to the target protein in a specific area called the active site (or active group). Typically, the

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active site is a pocket where the ligand may directly contact the nonpolar interior of the protein – an environment which excludes water. The result is a stable complex and the ability to carry out reactions which would not be possible in an aqueous solution.

Proteins are generally incapable of interaction in areas other than their active sites, since tight packing of polypeptide chains prevents penetration of random ligands. Nevertheless, the protein is not a monolith: its dynamic nature means that under certain conditions the packing of polypeptide chains may undergo relaxation, enabling small molecules to penetrate protein interior [1–6]. Those ligands cannot form stable bonds due to low binding energy in an area otherwise unprepared for specific interaction with such compounds – to put it simply, a rigid molecule is not likely to exhibit good alignment with the conformation of a folded polypeptide chain. The high mobility of small ligands also discourages strong interactions.

In spite of the above, some supramolecular associations of organic compounds are able to penetrate and anchor themselves inside proteins. This unique property emerges as a result of association (or self-association) of individual molecules [7–12], and is linked to the flexible structure and large interaction surfaces exposed by supramolecular ligands.

The presence of noncovalent bonds in supramolecular structures allows their components to shift with respect to one another, resulting in an adaptive ligand which has greater conformational alignment capabilities than polymers or small organic molecules.

Ongoing progress in supramolecular chemistry opens new research avenues and highlights new uses for associative structures [13–19]. Currently, research effort focuses primarily on technological improvements, including novel sieves, adsorption systems or tools which exploit various mechanical effects. The goal of such initiatives is to synthesize suitable monomers (or polymeric structures), which can then associate with one another according to a predefined blueprint, producing complex supramolecular units (Fig. 1.1). Relatively little work has been done in the area of identifying biological applications of such structures.

Noncovalent association as a means of generating complex structures is a ubiquitous phenomenon in nature. One classic example is the formation of molecular membranes, where a counterbalance of positive and negative charges in the polar component of each monomer eliminates electrostatic repulsion and allows molecules to align side by side in water, forming sheets. Another example involves microtubules which consist of self-associating proteins (Fig. 1.2).

Not all associative organic structures are capable of attaching to proteins. In fact, only one specific type of supramolecular structure can form complexes with sufficient stability to contemplate practical applications: systems which adopt ribbon-like micellar conformations (Fig. 1.3).

Ribbon-like micelles are the result of association of flat, polyaromatic, elongated, symmetrical molecules with polar groups at either end. Examples include CR and EB [20, 21]. Ligands consisting of several such molecules may penetrate into the protein interior by exploiting local instabilities or gaps created through accidental displacement of polypeptide loops. The ligand typically anchors itself between

Fig. 1.1 Formation of one-, two- and three-dimensional associations of monomers – schematic presentation

Fig. 1.2 Schematic depiction of cell membrane. Fencepost-like arrangement of phospholipid molecules enabled to close contact owing to charge neutrality. *Inset:* directed association of protein molecules – formation of microtubules

beta folds or random coils, since these two structural forms of polypeptide chain expose suitably large contact areas. Figure 1.4 illustrates the complexation process.

Owing to its structural flexibility, the supramolecular ligand may interact with proteins as the specific component – although its presence may also alter the target protein due to the large interaction area and strength of binding. Both structures adapt finally to each other, producing a stable bond [22].

Fig. 1.3 Formation of supramolecular ribbon-like CR micelle. **A** - trans form of CR, **B** - cis form of CR

The large volume of supramolecular ligands undoubtedly hampers penetration. Consequently, supramolecular ligands prefer interaction with inherently unstable proteins – such as partly unfolded proteins and amyloids [23–34]. In some cases, however, even a tightly packed protein may – when binding its natural target ligand – undergo sufficient structural rearrangement to permit penetration of additional large supramolecular ligands penetrating outside of the primary binding site. This type of interaction, while temporary, often drastically modifies the function of the protein [35, 36].

Asymmetrical bipolar molecules which form supramolecular systems in water, such as detergents, form also complexes with proteins by penetrating into their hydrophobic areas; however, this mode of interaction differs from the one used by symmetrical molecules. With detergents penetration is diffuse and occurs wherever low polarity is present. This process produces major changes in the protein's sec-

Fig. 1.4 CR/polypeptide complexation principle. (A and B) Molecular model (90-degree rotation). (C) Schematic view of CR (supramolecular) in complex with polylysine

ondary conformation, ultimately leading to denaturation. The unfolded skeleton of the protein is then reused by the supramolecular ligand as a seed for micellar aggregation. A typical example is the modification of polypeptide chains produced by SDS, commonly applied in polyacrylamide gel electrophoresis (Fig. 1.5) [37].

In contrast, flat, ribbon-like supramolecular ligands interact with proteins by “wedging” and produce no major changes in the protein’s distribution of hydrophobicity. Despite forming a complex with the ribbon-like ligand, the protein retains its native interaction capabilities. This effect is reinforced by the stability and cohesive nature of the ligand itself (caused by strong intermolecular association). If the target protein acquires the ability to bind a supramolecular structure as a result of interacting with its natural ligand, then the presence of the supramolecular structure tends to stabilize the original protein/ligand complex. This occurs in the case of nonreversible interactions, such as between antigens and antibodies. On the other hand, supramolecular ligands are also able to inhibit the activity of enzymes by “freezing” them in their complex with the substrate. Such uncompetitive inhibition differs by a mechanism from that known as noncompetitive one. It indicates that ribbon-like supramolecular structures may also be of use in pharmacology as distinct inhibitors [38, 39] (Fig. 1.6).

Fig. 1.5 Formation of rod-like structures of SDS: **A** - with protein backbone, **B** - without protein backbone

Fig. 1.6 Model view of enzyme inhibited by a supramolecular ligand (uncompetitive inhibition)

Uncompetitive inhibition is rarely encountered in nature as most inhibition processes are either competitive or noncompetitive in nature. In this specific case the inhibitor does not attach itself to the enzyme but rather to the entire enzyme-substrate complex, stabilizing it and negating the reversibility of complexation (Fig. 1.7).

It appears however that in order to form stable complexes with proteins without degrading their structure, supramolecular ligands must exhibit a ribbon-like conformation. It should also be noted that the distribution of polarity in a ribbon-like

Fig. 1.7 Mathematical formulation of uncompetitive inhibition

$V_{\max}$	Maximal rate
K_m	Michaelis constat
K_i	Dissotiation constat
$[S]$	-Substrate concentration
$[I]$	-Inhibitor concentration

$$v = \frac{V_{\max} [S]}{K_m + [S](1 + [I]/K_i)}$$

Fig. 1.8 Elongated association area of symmetric self-assembling molecules, necessary for formation of ribbon-like supramolecular structures

micelle approximates the properties of a beta fold, promoting formation of a stable complex.

A ribbon-like supramolecular structure may emerge only when the long axes of associating molecules are well aligned with each other. This condition is met when the axial alignment of each unit molecule is determined by its structural elongation (Fig. 1.8).

Another very important property of ribbon-like supramolecular ligands, promoting complexation, is the exposure of a large hydrophobic surface. The rigid structure and symmetrical distribution of charges in individual molecules prevent

Fig. 1.9 Exposure of non-polar fragments in a ribbon-like micelle composed of self-assembled symmetric molecules (arrow)

Fig. 1.10 Clouds of CR with solubilized, heat-aggregated IgG molecules (high molecular weight fraction of CR and heat-aggregated IgG complex extracted from molecular sieve chromatography – Sephadex G200). EM imaging (Reproduced by permission of *J. Physiology and Pharmacology*)

internalization of nonpolar fragments inside the micelle (which occurs in detergents). Such exposure of hydrophobicity on the ligand surface greatly enhances its complexation capabilities, and does so in a specific way: while promoting adhesion, it does not enable the ligand to independently penetrate into the protein – again, in contrast to detergents (Fig. 1.9).

Binding CR increases the protein's polarity, especially in light of the fact that, once anchored, a supramolecular ligand may sometimes propagate beyond the protein and attract additional dye molecules in its environment. As a result, a thermally aggregated protein (such as immunoglobulin G) may persist in solution, surrounded by free dye (Fig. 1.10) [40]. This has been proven through chromatographic separation (on a thin layer of Sephadex G200) of thermally aggregated immunoglobulin G solubilized in complex with the dye. Under EM imaging this heavy fraction appears as a cloud of dye particles with suspended thermally denatured immunoglobulin G, rendered soluble via complexation of CR.

Further adsorption of CR on Sephadex along the column eventually results in precipitation of insufficiently protected immunoglobulin molecules. To enhance the contrast of CR under EM we have added silver ions (AgNO_3), which form weak complexes with the dye but remain in solution along the short Sephadex filtration path.

1.2 Structural Adaptability of Molecules Forming Supramolecular Structures

Taken together, the presented characteristics – flat ribbon-like structure, flexibility, large interaction surface and exposure of hydrophobicity – promote interaction and formation of stable complexes with proteins penetrating to areas which are not biologically configured for binding ligands. Another important factor which enhances the adaptability of the CR micelle is some kind of plasticity of individual molecules, permitting rotation about the central bond between aromatic rings, as well as about both lateral azo bonds.

Substituents in conjugated chemical compounds – including polyaromatic compounds – act upon one another. This affects their properties as well as the properties of the entire molecule. The location of such substituents in the molecule is also important. Figure 1.11 presents potentiometric titration of CR and its derivative – 4,4'-bis(1-amino-6-sulphonaphthyl-4-azo-biphenyl Direct Red II), with an identical formula but a different arrangement of polar groups, resulting in a different value of amino groups – pK [41, 42].

Fig. 1.11 Altered placement of substituents in CR derivative resulting in altered pK of the amino group. Potentiometric titration. Control NaCl - red line

Fig. 1.12 Structural modification of CR molecule – formulas 1, 2, 3, 6 and EB – formulas 4, 5 and their effect on supramolecular binding to proteins: black bars – heat-aggregated IgG (less restrictive binding). Presented value – the number of bound molecules (molar ratio); gray bars – native IgG (more restrictive binding – antibodies agglutinating red cells). Presented value – enhancement of agglutination

The rotational freedom associated with azo bonds is strongly dependent on the substituents on aromatic rings; especially those located in close proximity to each azo bond and affecting its polarization, which may either enhance or stifle rotational freedom (Fig. 1.12). To illustrate this fact, we compare CR with its analogue – 4,4'-bis(1-amino-5-sulfonaphthyl-2-azo)biphenyl – where greater separation between the azo bonds and the sulfonic groups significantly reduces complexation capabilities (Fig. 1.12 – A and B). In contrast, fixation of the central bond in the fluorene derivative (with the accompanying planarization of the molecule) promotes

self-association, but reduces the system's flexibility. This is evidenced by more dense clustering and binding with thermally aggregated immunoglobulin G (where the ligand binding tolerance is high), but reduced capabilities for active site complexation in antibodies and the correspondingly weaker enhancement of agglutination (Fig. 1.12 – 1 and 3). Further analysis of this phenomenon is possible by measuring enhancement of agglutination in the SRBC-anti-SRBC model caused by complexation of CR. The observed effect is caused by greater involvement of serum polyclonal antibodies in agglutination resulting from complexation of the supramolecular ligand. Complexation capabilities are finally measured by: A – counting the number of dye molecules attached to a single thermally aggregated immunoglobulin G molecule where more than one anchoring site is present; B – assessing the degree to which agglutination is enhanced in the SRBC-anti-SRBC model, under the assumption that the supramolecular ligand increases antibody binding strength and its capacity for immunological complexation. Since such effects require precise alignment of the ligand with the domain V binding site, they provide a measure of the ligand's flexibility. This property can be directly quantified by measuring the readiness for immunological complexation of weak antibodies found in the polyclonal anti-SRBC serum, and the corresponding increase in agglutination.

The relation between degrees of rotational freedom, charge distribution and protein binding capabilities is also evident when comparing EB with Trypan blue (TB). Both dyes differ only with respect to the location of sulfonic groups. In Trypan blue this location is disadvantageous due to its proximity to both the azo bond and the central nonpolar region of the molecule (Fig. 1.12 – 4 and 5).

The need for a planar ribbon-like micelle becomes clear when we compare the complexation potency of CR with its derivative – 1,4-bis(1-amino-4-sulphonaphthyl-2-azo)phenylene, in which the central biphenyl group has been replaced with a benzene ring, eliminating the need for specific spatial orientation of the molecule's long axis. The resulting micelle is not a ribbon even though its unit molecule closely resembles CR (Fig. 1.12 – 1 and 6). Instead, it produces a self-associating cylindrical structure, protecting nonpolar fragments from the external environment [43]. This effect also negates the protein complexation capabilities of such a ligand (Fig. 1.13). Comparing the properties of both structurally similar dyes highlights the need for a ribbon-like conformation in supramolecular protein ligands.

Regarding protein structures, supramolecular ligands tend to preferentially form complexes with beta-structure and random coils of polypeptide chains. Elongated non-helical polypeptides represent a good match for the supramolecular ribbon itself, providing the ligand with a convenient anchoring point (Fig. 1.4).

Susceptibility for supramolecular ligand penetration varies from protein to protein. In addition to the degree of packing and the protein's intrinsic stability it also depends – as remarked above – upon function-related conformational rearrangement [35, 36, 38, 39].

The role of most proteins is to interact with specific targets. Such interaction affects the protein itself and often results in partial unfolding, which renders the protein susceptible to further penetration by a supramolecular ligand. This mechanism can be observed e.g. when analyzing the interaction of CR with

Fig. 1.13 Self-assembly of molecules without imposed orientation (lack of elongated contact area), resulting in a cylindrical supramolecular structure with internalized non-polar fragments. Chemical formula of 1,4-bis(1-amino-4-sulphonaphtyl-2-azo)phenylene

serum proteins. Note that the bloodstream typically contains acute phase proteins, in complex with their respective ligands, and that such complexes are recognized and eliminated by macrophages and liver enzymes. We may therefore suspect that the capability for such selective elimination depends on function-related structural changes which occur in proteins introducing some local instability.

1.3 Specificity of Congo Red Complexation

The complexation capabilities of CR increase along with the dye's concentration. This is related to increased probability of penetration into proteins as they undergo dynamic – and often temporary – structural changes. Furthermore, increased concentrations favor supramolecular association, resulting in longer micelles with more pronounced dipole characteristics. This effect can be revealed by measuring electrophoretic migration distance on the electrophoretic plate and present it as a function of dye concentration.

It seems that the properties of CR change qualitatively as its concentration increases, favoring protein complexation. At high concentrations the dye is even capable of penetrating into proteins at room temperature. The application of DMSO results in dissociation of the supramolecular structure; consequently, the electrophoretic migration speed again becomes independent of concentration (Fig. 1.14).

Fig. 1.14 Increased electrophoretic migration rate of CR corresponding to increased dye concentration: **A** - 1,2,3,4,5, position 6 - bromophenol blue dye. **B** - concentration-independent migration in (DMSO – buffer mixture 1:2). Evidence of close CR self-assembly

Fig. 1.15 Two mutually-related complexes consisting of L-lambda chains and CR molecules, exhibiting different migration rates in electrophoresis (*b* and *c*) due to different well defined dye load. *a* – L chain, *b* – L chain complexed with 4 molecular CR ligand, *c* – L chain complexed with 5–8 molecular CR ligand, *d* – CR excess. Complexation induced by the step-wise increased concentration of CR - 1,2,3,4

This proves that concentration determines the emergent, supramolecular properties of the dye – particularly its capability to form stable complexes with proteins.

In order to demonstrate this phenomenon, we have selected an immunoglobulin light chain which is relatively resistant to CR complexation in its native form. Heating the protein promotes complexation, but even at room temperature high concentrations result in two distinct complexes which migrate faster than the base protein – depending on the number of ligand molecules present in each complex. Here, complexation involves the variable V domain (Fig. 1.15) [44]. The “slow” migrating fraction carries ligands composed of four dye molecules, while in the “fast” fraction

Fig. 1.16 Spectral shift of CR in a low-polarity environment – **A** – 1 – native concanavalin, 2 – heated concanavalin) and **B** – in alcohols (1 – native concanavalin 2-methanol, 3-ethanol, 4-propanol) characterized by varying polarity

the size of the ligand varies between 5 and 8 molecules. Notably, the “fast” fraction produces a smeared electrophoretic band, showing that the ligand grows over time and that larger complexes are produced with greater difficulty than smaller ones.

The supramolecular ligand forms a tight bond with the protein and adapts itself to the new environment, as evidenced by a spectral shift towards greater wavelengths. This effect results from transitioning between water and an environment characterized by lower values of the dielectric constant (Fig. 1.16A) [45, 46].

Fig. 1.17 Dye protected against acidification by complexed protein measured by spectral change. 1 – free dye, 2 – dye bound to protein (L chain IgG)

Fig. 1.18 Dye protected against reduction by complexed protein measured by spectral change. 1 – dye bound to protein, 2 – free dye

To further illustrate this effect, the spectrum of CR has been analyzed in the presence of alcohols containing increasingly larger nonpolar components: methanol, ethanol and propanol. The observed shift towards greater wavelengths confirms the stated hypothesis (Fig. 1.16B).

A complexed ligand bound in protein interior is protected against acidification by the protein. CR changes its color in an acidic environment due to change in the ionization of its amino groups. The color shift from red to blue is decisive and rapid in unbound dye, whereas dye-protein complexes retain their red coloration for some time (Fig. 1.17). Reduction by sodium dithionite deprives the dye of its color due to cleavage of azo bonds; however, protein complexation slows this reaction down significantly (Fig. 1.18).

Fig. 1.19 Self-assembly tendency correlated with corresponding different dye complexation activity measured as the yield of dye protein complexation at increasing temperature. Lines 1 and 2 according to chemical formulas 1 and 2 respectively. 1 - CR, 2 - 1,4-bis(1-amino-4-sulphonaphthyl-2-azo) phenylene

In summary, complexes consisting of supramolecular dyes and proteins appear to result from penetration of associated dye molecules into the target protein. The capability for such penetration depends on the cohesiveness of the dye micelle, as well as its shape.

In order for a supramolecular aggregation to function as a distinct protein ligand, the unit composed of self-assembled molecules must behave as a coherent whole. This depends on the self-association potency of the target substance – powerful self-association produces a ligand which readily interacts with proteins (Figs. 1.19 and 1.20).

Another important property of supramolecular dyes is their capability to intercalate foreign bodies (other than the self-associating unit molecules), resulting in ligands which can introduce foreign substances into proteins even when the protein does not, by itself, react with such substances [17, 47]. Rhodamine B – a basic dye which exhibits strong fluorescence and is therefore useful in imaging studies – may be intercalated into CR micelles and bound to proteins. Other potential intercalants

Fig. 1.20 Efficiency of formation the protein-dye complex corresponding to self-assembly tendency – registered at increasing temperatures. Lines 1 and 2 correspond to chemical molecules of chemical formulas 1 (EB) and 2 respectively (TB)

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include heavy metal ions – such as in the case of TY, used as a carrier for silver ions to provide contrast for EM imaging of amyloid deposits [48]. The same mechanism may be used to introduce some alterations to properties of proteins.

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References

1. Kay LE (1998) Protein dynamics from NMR. *Biochem Cell Biol* 76(2–3):145–152
2. Doyle DA, Lee A, Lewis J, Kim E, Sheng M, MacKinnon R (1996) Crystal structures of a complexed and peptide-free membrane protein-binding domain: molecular basis of peptide recognition by PDZ. *Cell* 85(7):1067–1076

3. Fuentes EJ, Der CJ, Lee AL (2004) Ligand-dependent dynamics and intramolecular signalling in a PDZ domain. *J Mol Biol* 335(4):1105–1115
4. Fraser JS, Clarkson MW, Degnan SC, Erion R, Kern D, Alber T (2009) Hidden alternative structures of proline isomerase essential for catalysis. *Nature* 462(7273):669–673
5. McLaughlin RN Jr, Poelwijk FJ, Raman A, Gosal WS, Ranganathan R (2012) The spatial architecture of protein function and adaptation. *Nature* 491(7422):138–142
6. Laskowski RA, Gerick F, Thornton JM (2009) The structural basis of allosteric regulation in proteins. *FEBS Lett* 583(11):1692–1698
7. Gunasekaran K, Ma B, Nussinov R (2004) Is allostery an intrinsic property of all dynamic proteins? *Proteins* 57(3):433–443
8. Kern D, Zwietering ER (2003) The role of dynamics in allosteric regulation. *Curr Opin Struct Biol* 13(6):748–757
9. Tompa P (2011) Unstructural biology coming of age. *Curr Opin Struct Biol* 21(3):419–425
10. Wright PE, Dyson HJ (1999) Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. *J Mol Biol* 293(2):321–331
11. England JL (2011) Allostery in protein domains reflects a balance of steric and hydrophobic effects. *Structure* 19(7):967–975
12. Koshland DE Jr (1959) Enzyme flexibility and enzyme action. *J Cell Comp Physiol* 54:245–258
13. Zeng C, Chen Y, Kirschbaum K, Lambright KJ, Jin R (2016) Emergence of hierarchical structural complexities in nanoparticles and their assembly. *Science* 354(6319):1580–1584
14. Liu W, Tagawa M, Xin HL, Wang T, Emamy H, Li H, Yager KG, Starr FW, Tkachenko AV, Gang O (2016) Diamond family of nanoparticle superlattices. *Science* 351(6273):582–586
15. Sacanna S, Irvine WT, Chaikin PM, Pine DJ (2010) Lock and key colloids. *Nature* 464(7288):575–578
16. Desiraju GR (2001) Chemistry beyond the molecule. *Nature* 412(6845):397–400
17. Swanson BD, Sorensen LB (1995) What forces bind liquid crystals? *Phys Rev Lett* 75(18):3293–3296
18. Herzfeld J (1996) Entropically driven order in crowded solutions: from liquid crystals to cell biology. *Acc Chem Res* 29(1):31–37
19. Lv JA, Liu Y, Wei J, Chen E, Qin L, Yu Y (2016) Photocontrol of fluid slugs in liquid crystal polymer microactuators. *Nature* 537(7619):179–184
20. Evers CH, Luiken JA, Bolhuis PG, Kegel WK (2016) Self-assembly of microcapsules via colloidal bond hybridization and anisotropy. *Nature* 534(7607):364–368
21. Skowronek M, Stopa B, Konieczny L, Rybarska J, Piekarska B, Szneler E, Bakalarski G, Roterman I (1998) Self-assembly of Congo red – a theoretical approach to identify its supra-molecular organization in water and salt solutions. *Biopolymers* 46:267–281
22. Król M, Roterman I, Piekarska B, Konieczny L, Rybarska J, Stopa B, Spólnik P, Szneler E (2005) An approach to understand the complexation of supramolecular dye Congo red with immunoglobulin L chain lambda. *Biopolymers* 77(3):155–162
23. Stopa B, Rybarska J, Drozd A, Konieczny L, Król M, Lisowski M, Piekarska B, Roterman I, Spólnik P, Zemanek G (2006) Albumin binds self-assembling dyes as specific polymolecular ligands. *Int J Biol Macromol* 40(1):1–8
24. Edelman GM, Gally JA (1962) The nature of Bence-Jones proteins. Chemical similarities to polypeptide chains of myeloma globulins and normal gamma-globulins. *J Exp Med* 116:207–227
25. Nakano T, Matsui M, Inoue I, Awata T, Katayama S, Murakoshi T (2011) Free immunoglobulin light chain: its biology and implications in diseases. *Clin Chim Acta* 412(11–12):843–849
26. Leitzgen K, Knittler MR, Haas IG (1997) Assembly of immunoglobulin light chains as a prerequisite for secretion. A model for oligomerization-dependent subunit folding. *J Biol Chem* 272(5):3117–3123
27. Kaplan B, Livneh A, Sela BA (2011) Immunoglobulin free light chain dimers in human diseases. *Sci World J* 11:726–735

28. Charafeddine KM, Jabbour MN, Kadi RH, Daher RT (2012) Extended use of serum free light chain as a biomarker in lymphoproliferative disorders: a comprehensive review. *Am J Clin Pathol* 137(6):890–897
29. Woodcock S, Henrissat B, Sugiyama J (1995) Docking of congo red to the surface of crystalline cellulose using molecular mechanics. *Biopolymers* 36(2):201–210
30. Khurana R, Gillespie JR, Talapatra A, Minert LJ, Ionescu-Zanetti C, Millett I, Fink AL (2001) Partially folded intermediates as critical precursors of light chain amyloid fibrils and amorphous aggregates. *Biochemistry* 40(12):3525–3535
31. Howie AJ, Brewer DB (2009) Optical properties of amyloid stained by Congo red: history and mechanisms. *Micron* 40(3):285–301
32. Buell AK, Dobson CM, Knowles TP, Welland ME (2010) Interactions between amyloidophilic dyes and their relevance to studies of amyloid inhibitors. *Biophys J* 99(10):3492–3497
33. Wang Y, Liu Y, Deng X, Cong Y, Jiang X (2016) Peptidic β -sheet binding with Congo Red allows both reduction of error variance and signal amplification for immunoassays. *Biosens Bioelectron* 86:211–218
34. Frid P, Anisimov SV, Popovic N (2007) Congo red and protein aggregation in neurodegenerative diseases. *Brain Res Rev* 53(1):135–160
35. Lendel C, Bolognesi B, Wahlström A, Dobson CM, Gräslund A (2010) Detergent-like interaction of Congo red with the amyloid beta peptide. *Biochemistry* 49(7):1358–1360
36. Rybarska J, Konieczny L, Roterman I, Piekarska B (1991) The effect of azo dyes on the formation of immune complexes. *Arch Immunol Ther Exp* 39(3):317–327
37. Jagusiak A, Konieczny L, Krol M, Marszalek P, Piekarska B, Piwowar P, Roterman I, Rybarska J, Stopa B, Zemanek G (2015) Intramolecular immunological signal hypothesis revived-structural background of signalling revealed by using Congo Red as a specific tool. *Mini Rev Med Chem* 4(13):1104–1113
38. Weber K, Osborn M (1969) The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J Biol Chem* 244(16):4406–4412
39. Kaszuba J, Konieczny L, Piekarska B, Roterman I, Rybarska J (1993) Bis-azo dyes interference with effector activation of antibodies. *J Physiol Pharmacol* 44(3):233–242
40. Shrestha S, Shim YS, Kim KC, Lee KH, Cho H (2004) Evans Blue and other dyes as protein tyrosine phosphatase inhibitors. *Bioorg Med Chem Lett* 14(8):1923–1926
41. Piekarska B, Konieczny L, Rybarska J, Stopa B, Spólnik P, Roterman I, Król M (2004) Intramolecular signaling in immunoglobulins – new evidence emerging from the use of supramolecular protein ligands. *J Physiol Pharmacol* 55(3):487–501
42. Stopa B, Piekarska B, Konieczny L, Rybarska J, Spólnik P, Zemanek G, Roterman I, Król M (2003) The structure and protein binding of amyloid-specific dye reagents. *Acta Biochim Pol* 50(4):1213–1227
43. Spólnik P, Konieczny L, Piekarska B, Rybarska J, Stopa B, Zemanek G, Król M, Roterman I (2004) Instability of monoclonal myeloma protein may be identified as susceptibility to penetration and binding by newly synthesized Congo red derivatives. *Biochimie* 86(6):397–401
44. Zemanek G, Konieczny L, Piekarska B, Rybarska J, Stopa B, Spólnik P, Urbanowicz B, Nowak M, Król M, Roterman I (2002) Egg yolk platelet proteins from *Xenopus laevis* are amyloidogenic. *Folia Histochem Cytobiol* 40(3):311–318
45. Piekarska B, Konieczny L, Rybarska J, Stopa B, Zemanek G, Szneler E, Król M, Nowak M, Roterman I (2001) Heat-induced formation of a specific binding site for self-assembled Congo Red in the V domain of immunoglobulin L chain lambda. *Biopolymers* 59(6):446–456
46. Piekarska B, Konieczny L, Rybarska J, Stopa B, Zemanek G, Szneler E, Król M, Nowak M, Roterman I (2001) Heat-induced formation of a specific binding site for self-assembled Congo Red in the V domain of immunoglobulin L chain lambda. *Biopolymers* 9(6):446–456

47. Konieczny L, Piekarska B, Rybarska J, Stopa B, Krzykwa B, Noworolski J, Pawlicki R, Roterman I (1994) Bis azo dye liquid crystalline micelles as possible drug carriers in immunotargeting technique. *J Physiol Pharmacol* 45(3):441–454
48. Konieczny L, Piekarska B, Rybarska J, Skowronek M, Stopa B, Tabor B, Dabroś W, Pawlicki R, Roterman I (1997) The use of Congo red as a lyotropic liquid crystal to carry stains in a model immunotargeting system – microscopic studies. *Folia Histochem Cytobiol* 35(4):203–210

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