

1 **Network organization of antibody interactions in sequence and structure space: the**
2 **RADARS model**

3

4

5 József Prechl

6

7 Diagnosticum zrt, Attila u. 126, Budapest, Hungary

8

9 Correspondence: jprechl@gmail.com

10

11

12

13 **Keywords:** antibody; network; sequence; structure; clonality; B cell; systems biology;
14 quantitative biology; immunodominance; consecutive reactions; energy partition

15 **Abstract**

16

17 Adaptive immunity in vertebrates represents a complex self-organizing network of protein
18 interactions that develops throughout the lifetime of an individual. While deep sequencing of
19 the antibody repertoire may reveal clonal relationships, functional interpretation of such data
20 is hampered by the inherent limitations of converting sequence to structure to function. In this
21 paper a novel model of antibody interaction space and network, termed radial adjustment of
22 system resolution, or RADARS, is proposed. The model is based on the radial growth of
23 interaction affinity of antibodies towards an infinity of directions representing molecular
24 shapes. Levels of interaction strength appear as energy shells of the system. B-cell development
25 and immune responses are interpreted in the model and quantitative properties of the antibody
26 network are inferred from the physical properties of a quasi-spherical system growing multi-
27 radially. The concept of system equilibrium constant is introduced, which is the median of
28 equilibrium constants in the system and serves to define probability of interactions. This
29 thermodynamic system is described by a power-law distribution of antibody free energies with
30 a network degree exponent of ϕ^2 , representing a scale-free network of antibody
31 interactions. Plasma cells are network hubs, memory B cells are nodes with intermediate
32 degrees and B1 cells represent nodes with minimal degree. As an energy transduction system
33 this network serves to optimize free energy consumption, removing antigens at the required
34 rate at the same time.

35 Thus, the RADARS model implies that an absolute sequence space is reduced to a
36 thermodynamically viable structure space by means of a network of interactions, which control
37 B-cell development. Understanding such quantitative network properties of the system should
38 help the organization of sequence-derived structural data, offering the possibility to relate
39 sequence to function in a complex, self-organizing biological system.

40

41 **1. Introduction**

42

43 Appearance of complex multicellular life was accompanied by the evolution of a system that
44 maintains cellular and molecular integrity in the host organism (1). The adaptive immune
45 system is a complex system in the physical sense, being composed of a vast number of cells
46 that engage in interactions, self-organize and - most impressively - adapt to the molecular and
47 cellular environment. Its mere size, with cell numbers in the range of 10^{11} , suggests that system
48 could be described by statistical properties. In fact, the host is more than an organism: a
49 supraorganism (2,3) with microbial communities, and immunity maintains a continuity of
50 interactions rather than simply discriminating self from non-self (4). Technological advances
51 now allow us to measure and characterize this complexity in ever growing details, at the gene,
52 transcript, protein and cellular levels, driving the field of systems immunology (5). The vast
53 amount of data generated requires not only data storage and analysis capacity, but also
54 theoretical frameworks, models that simplify data organization and systems level
55 interpretation.

56 Humoral adaptive immunity comprises the cells and mechanisms that lead to the production of
57 antibodies. In human adults B-cells develop in the bone marrow throughout life and build up a
58 system of effector and memory cells, which accumulate as a lifetime of immunological
59 experiences. Continuously emerging naive B cells only differentiate further if selected for
60 immunological actions based on their B-cell antigen receptor (BCR) specificity. The primary
61 role of B cells and antibodies is the regulation of antigen removal and thereby antigen
62 concentration in the host, operating over several orders of magnitude(6). Because this
63 specificity is genetically coded in the individually rearranged immunoglobulin heavy and light
64 chain sequences, it is possible to capture the antibody repertoire in a given sample of B cells.
65 Deep sequencing or next generation sequencing (NGS) is capable of generating sequence data
66 of antibody repertoires with varying resolution and length (7–11).

67 It is also possible to profile the antibody repertoire functionally, based on the identification of
68 antibodies binding to huge sets of potential targets (12,13). This approach is biased by the fact
69 that a priori knowledge of targets is not always possible and only those antibodies that bind to
70 the tested antigens are identified. Antigen microarray assays are useful for the focused analysis
71 of antibodies related to allergy, autoimmunity, infection or cancer (14–18). Such functional
72 analyses provide a more meaningful profile in the immunological sense and if carried out from
73 blood it is less prone to sampling error than cell-based sequencing approaches.

74 The relationship between antibody sequence and structure is on one hand like that of proteins
75 in general: polypeptide chains of a given sequence fold into structures, which are responsible
76 for function. In the enormous sequence space allowed by permutating amino acids only the
77 thermodynamically stable structures materialize as proteins (19). Proteins capable of
78 interacting with molecules in a way that improves chances of survival of the host organism will
79 themselves survive and evolve. Unlike proteins in general, antibodies evolve within the lifetime
80 of the host. While thermodynamic constraints still hold, their “survival”, meaning the
81 producing cell clone being selected into long-lived B-cell populations, is determined by
82 interactions with self and foreign molecules, the antigens. Importantly, because there are so
83 many more sequences than structures and because changing a few critical amino acids can
84 result in different structures, mapping sequence space to structure space is far from trivial. The
85 combined length of the complementarity determining regions (CDR) of heavy and light
86 immunoglobulin chains is around 14-50 amino acids (IMGT definition (20)). By employing
87 screening and selection mechanisms, coupled with cycles of random mutagenesis, targeting
88 primarily these amino acids, the immune system is capable of developing high-affinity binders
89 against most targets. Understanding these processes on the systems level preferably requires
90 the prediction of structures from NGS data (21) because of the complex sequence-to-space
91 relationship, as noted above. The architecture and functioning of complex systems can be
92 assessed by network science, which in the case of antibodies identifies antibody-antigen
93 interaction networks (22). The development of concepts of the immune system as a network
94 were key steps in our current perception of immunity (23,24). Efforts are now under way to
95 describe the immune system as a network (termed network systems immunology) using NGS
96 data and network science (25). Since the system is organized by structure rather than sequence,
97 the conceptualization of an antibody interaction network based on physical properties should
98 help better definition of the system.

99 In this paper, following a brief introduction to the sequence space of antibodies, a model for
100 the molecular organization of antibody structure space or interaction space is proposed. The
101 model builds on the generalized quantitative model of antibody homeostasis (6,26,27), thus
102 approaches antibody function from the physico-chemical perspective: antibodies are organized
103 into a network by binding affinity to cognate target. The model also considers the architecture
104 of B-cell development and hierarchy and provides a power law-based quantitative network
105 description of the humoral immune system.

106

107 **2. Antibody clonal network representation in sequence space**

108

109 Sequence space in the biological sense is a theoretical space comprising collections of nucleic
110 acid or protein sequences of interest. We usually talk about protein sequence space and define
111 what protein sets are involved (proteome of a given species, cells, etc.) and whether any
112 restrictions hold (fully random, functional, identified, etc.). An amino acid sequence with a
113 given length 'd' and full randomization with 20 amino acids occupies a sequence space 20^d
114 (Fig 1A). An exact sequence with no ambiguity defines an exact position in sequence space;
115 moves in this space are discrete steps along a given dimension. As the figure suggests, it is
116 impossible to visualize high-dimensional protein space in 2D. Exponential growth is incredibly
117 fast, leading to the generation of vast amounts of space in high dimensions.

118 It is accepted that only a fraction of all theoretically possible sequences are thermodynamically
119 stable and protein evolution can be interpreted as a search for acceptable and functional
120 structures in sequence and structure space (19). Thinking along these lines, the evolution of
121 antibody binding surface, the paratope, is a search for the thermodynamically stable sequences
122 and the selection from among these the ones meeting immunological criteria for B-cell survival.
123 The set of viable antibody sequences, functional antibody sequence space, lies much below the
124 theoretically possible (28) and close to the already observed and annotated antibody sequence
125 space (29).

126 Collections of antibody protein sequences obtained by translating DNA or RNA of deep
127 sequencing data ideally span the whole variable domain of heavy (VH) and light chains (VL)
128 and can also pair these two. In such a case the gene segments contributing to the rearrangement
129 of VH and VL can be predicted and visualized in 3D and 2D respectively, as shown (Figure
130 1B). A repertoire can be represented by identifying coordinates of rearrangements identified,
131 and symbol size or color can represent segment frequencies (30). While the use of gene
132 segments for classification allows tremendous reduction in dimensionality, it is not best suited
133 for functional network analysis, where the use of complete rearranged and mutated sequences
134 is preferable (31).

135 In a much simpler approach, heavy chain CDR3 regions only are used as an estimate of
136 diversity. Though this region is often regarded as being most important for determining binding
137 specificity, identical H-CDR3 sequences have been found to be present in functionally
138 unrelated cells and therefore H-CDR3 seems insufficient for functional classification (32).
139 Selection of the pre-BCR bearing cells depends on signals that may be triggered by ubiquitous
140 ligands present in the bone marrow microenvironment. The presence of uniform reactivity

141 against such common public self-antigens may lead to the positive selection of CDR3 with
142 similar binding properties, and thereby similar sequences. Sequencing of the complete heavy
143 chain variable domains can be readily used to follow changes in repertoire size and diversity
144 during B-cell development and response to immunization (33).

145 Whatever the depth and methodology, sequence similarity relationships can be used for the
146 construction of family trees, often displayed in circular forms. These trees usually start
147 classification with the V segment, clustering clones with common V use (34). While this
148 approach may be useful for classification, the use of the complete VDJ-H sequence as a first
149 stage classifier, followed by VJ-L use better reflects the natural development scheme of B cells
150 (Fig 1C). Antibody repertoire sequencing now follows guidelines to help the integration of data
151 (35–37), several tools and databases devoted especially for these data have been established
152 (8,10,38–40).

153

154 **3. Antibody interaction space representation in structure space**

155 In contrast to this graded qualitative scheme, which may well serve the purpose of tracking
156 peripheral clonal expansions accompanied by affinity maturation, a quantitative scheme should
157 place genetic changes into structure rather than sequence space. Furthermore, because it is not
158 just antibody structure but also the availability of targets and the structure of those targets that
159 determine the development of antibody repertoire and the architecture of the network, we shall
160 talk about interaction space, as explained below.

161 *3.1. Structural resolution of molecular recognition as a measure of interaction strength*

162 While sequence can be defined with various levels of certainty of an amino acid occupying a
163 given position in the sequence, molecular structure can be defined at various levels of
164 resolution. As we are talking about antibody molecules structural resolution is on the atomic
165 scale, crystal structures define atomic coordinates on the Ångstrom scale. The binding site of
166 an antibody can also be characterized by the surface area that comes into close contact with the
167 antigen (41,42). Water molecules are displaced from this area as a function of the goodness of
168 fit. The so-called buried surface area (BSA) is therefore a predictor of binding energy of protein
169 interactions (43,44). Another measure of goodness of fit is the decrease of free energy of the
170 antibody molecule upon binding. All these approaches are correlated: higher resolution
171 “description” of a structure by the antibody corresponds to greater BSA and to a higher binding
172 energy. In other words, the resolution of molecular recognition is the goodness of fit in terms
173 of number and strength of non-covalent bonds forming between antibody and target and can

174 be expressed as standard free energy change ΔG° or as equilibrium constant K of binding.

175 These two are related by the equation

176 $\Delta G^\circ = -RT \ln K$ 1)

177 alternatively

178 $K = \exp(-\Delta G^\circ / RT)$ 2)

179 where R is the product of k_B , the Boltzmann constant, and N_A , the Avogadro number, and T
180 is thermodynamic temperature in Kelvins. The Boltzmann constant serves to relate the energy
181 of particles to temperature, N_A expresses energy per mole particles. Considering that the
182 recognition of a given antigen A_g ; is changing in the system by the adjustment of Ab fit, we
183 can characterize this maturation of affinity by examining the free energy level of the antibody,
184 such as

185 $\Delta G^\circ = F_b - F_f$ 3)

186 where F_b is the free energy of the bound (also called native) and F_f is the free (non-native)
187 form of the antibody; ΔG° is the Gibbs free energy of formation of 1 mole antibody under
188 standard conditions.

189 The advantage of using thermodynamic description for the characterization of structural
190 resolution is that it conveys the sense of function: higher binding energy means higher affinity
191 of antibody to target, which in turn means more efficient clearance (6). Besides defining
192 resolution of molecular recognition, which is a general descriptor, the identification of a given
193 interaction requires the description of target shape, a distinct molecular structure. The higher
194 the resolution the more information is required for defining shape, translating into a better fit
195 between antibody and target.

196 By starting to increase resolution we shall be able to distinguish between different shapes, the
197 higher the resolution the more shapes becoming distinct. Because of the structural complexity
198 of an antibody binding surface area, the distinction between all possible shapes at high
199 resolution would require a multidimensional space. Let us gradually generate a
200 multidimensional interaction space by considering a point of origin, the center of the system,
201 from which a particular direction represents a particular shape. In this representation the extent
202 by which we leave the point of origin corresponds to the resolution at which we can define the
203 direction. Thus, going away from the minimal resolution we can define shape at gradually
204 higher resolutions, corresponding to larger free energy decrease of the interacting molecule
205 (Fig. 2A,B). Different levels of resolution, that is different levels of binding energies appear in
206 our scheme as shells of a sphere. Theoretically the number of directions originating from a
207 single point is infinite, so the shapes available in this representation are also infinite if we go

208 far enough. The other way around, higher resolution of molecular recognition is required to
209 distinguish two similar antigenic structures. Practically, considering a reversible interaction,
210 the resolution is limited by the binding energy of reversible interactions.

211 This model of the organization of interactions of a system we shall call ‘RADial ADjustment
212 of System Resolution’ or RADARS in short. The abbreviation intentionally reminds of
213 radiolocation, where emitted electromagnetic waves interact with objects in their way and are
214 reflected to provide an image of the surroundings. The RADARS model implies that elements
215 of the growing system interact with the surroundings, gaining information and adjusting system
216 growth accordingly.

217 *3.2. B-cell development in interaction space*

218 Immunological interpretation of the model requires us to fit B-cell development and antibody
219 network evolution into this interaction space. We shall assume that a common lymphoid
220 progenitor has the potential to generate any and all functional VDJ-VJ sequences and therefore
221 to produce via sequential differentiation and maturations steps antibody against any and all
222 targets. By functional VDJ-VJ sequences we mean all sequences that are physically and
223 biologically viable. This means thermodynamic stability (able to fold into a structure
224 compatible with the Ig domain), ability to pair, forming a VH-VL functional binding unit, and
225 ability to sustain a B-cell via delivering survival, differentiation and proliferation
226 signals(26,45).

227 A differentiation step that reduces this total potential introduces restrictions in structural space.
228 This will appear as a step towards increased resolution of cognate target structure recognition.
229 Expression of the surrogate light chain (SLC) marks the first step towards BCR formation.
230 These pro-B cells represent the founders of all B cells (Fig. 2C). While signaling via the SLC
231 may be required, it is uncertain whether binding of SLC is required for further differentiation,
232 therefore we assume that these cells seed the complete antibody interaction space.
233 Rearrangement of the heavy chain introduces a structural restriction: a particular functional
234 heavy chain variable domain (VH) sequence has a limited range of targets. Pre-B cells
235 displaying the pre-BCR composed of VH-SLC pairs will divide until and as long as ligands
236 (antigens) are available. Cells with different VH sequences will populate the structural space
237 and share this space according to the availability and in the direction of target. Cells with more
238 abundant targets expand more, cells with less frequent targets remain in lower numbers, until
239 optimal BCR engagement is achieved (26). As a result, interaction space as represented at these
240 resolutions will be filled with different pre-B cell clones according to the availability of the
241 common self-antigens.

242 The next levels of interaction resolution, introducing further focusing in interaction space,
243 comes with the rearrangement of the light chain. Individual pre-B cells rearrange their light
244 chains independently and randomly. Therefore, all pre-B cells reserve a particular area on the
245 next level of structural resolution. The size of this area again will correspond to the nature of
246 the rearranged VL domain, with those finding more available targets expanding more. The pool
247 of immature B cells fills thus the outer level of resolution in the bone marrow (Fig. 2C).

248 Taking a somewhat unique route of differentiation are the B1 cells. These cells seem to generate
249 antibodies that keep B1 cells in a continuous state of low-level activation. This may reflect
250 their ability to respond to soluble, highly abundant antigens (26), or an intrinsic ability of the
251 BCR of sustained signaling, perhaps due to structural properties (46). In any case, B1 cells
252 represent a stable population with the highest affinity towards self and non-self, which is
253 achieved without affinity maturation. Meanwhile B2 cells are continuously generated but die
254 in a few days unless recruited as effector cells for further differentiation (Fig. 2C).

255 To allow for an even balance between differentiation and division we can employ a Fibonacci
256 branching scheme in our model: every division yields one cell that divides again and another
257 one that differentiates, changing its BCR (Fig. 2C). Assuming equal time and energy
258 investments for division and differentiation this scheme results in a Fibonacci series number of
259 cells with distinct BCR after every time unit. In our model dividing cells remain on the same
260 level of resolution, differentiating cells move outwards if interaction resolution grows, in a
261 hierarchy similar to that proposed by Derényi&Szöllősi (47). To maintain constant cell
262 numbers at all levels of differentiation the ratio of differentiation rates is proposed to be
263 determined by ϕ , the number approached by Fibonacci series. Thus, on a systemic level, for
264 every new incoming cell there will be one differentiated cell entering the next level, along with
265 a differentiated cell already dividing on the next level (Fig. 2C inset). In our model this also
266 means a ϕ^2 increase in surface area to be filled with differentiated cells continuously
267 replenished from lower levels, along with a proportional decrease of directions (shapes)
268 available for a given cell.

269 As implied above, selection in the bone marrow is a passive process: randomly generated
270 sequences find their positions in the structural space, expanding according to the availability
271 of interacting target structures. As a result, the emerging population of immature B cells will
272 bear the low-resolution antigenic signature of the bone marrow environment. This can be
273 interpreted both as deletion of highly self-reactive clones to prevent autoimmunity (45), and as

274 selection for mildly autoreactive clones to help homeostatic antibody functions and setting a
275 reference for recognition of non-self (48).

276 *3.3. Immune responses in interaction space*

277 The development of cells reacting with antigens that are only temporarily present in the host
278 presents the risk of investing energy into clones that will become useless once that antigen
279 disappears. Therefore, such clonal expansions beyond the border of self only takes place when
280 2nd signals inform the host of danger. This is the development of an immune response, aided
281 by various molecular and cellular help signals. Thymus independent and primary thymus
282 dependent responses expand populations of B cells without improving their affinity, thus
283 keeping them on the same level in the interaction space. We shall call these cells effector B
284 cells. Thymus dependent responses aided by helper T cells lead to the formation of germinal
285 centers where affinity maturation takes place. This adjustment of affinity focuses interactions
286 into a particular direction, corresponding to molecular shape in our model, leading to increased
287 resolution of interaction space only in that direction. Post-germinal center B cells will have
288 accumulated somatic hypermutations to increase their affinity. In this region of interaction
289 space, affinity and corresponding resolution of target recognition is high, but once target is
290 cleared cells go into dormancy. These are the memory B cells that conserve the genetic
291 information acquired during affinity maturation but minimize their activity: no divisions, no
292 antibody secretion; remaining guards against future encounter with the same or similar target
293 (Fig. 2C) (49–51). Another type of cell that remains in the system after target has been cleared
294 is plasma cell, which become long-lived antibody producing cells following terminal
295 differentiation (52,53).

296

297 **4. Characterization of the antibody interaction network**

298 *4.1 Distribution of binding energies in the system*

299 The cell biological aspects of the RADARS model are summarized in Figure 3A. Expansion
300 and differentiation of cells originating from CLPs create a cellular repertoire in the bone
301 marrow, then further expansions and differentiation steps increase this repertoire and
302 supplement it with antibody secretion. The development of B cells bearing surface antibodies,
303 B-cell receptors, with increasing affinities takes place in an environment loaded with a huge
304 diversity of macromolecules. These antibodies thus develop in a system characterized by
305 constant reversible, non-covalent interactions. These interactions in the system can be
306 described mathematically by the frequency distribution of interaction affinity. According to the
307 random energy model the free energy of biomolecular interactions is normally distributed(54).

308 Testing a given antigen against a universe of randomly generated antibodies would yield a
309 normal distribution of binding energies. However, it is exactly the role of the immune system
310 to adjust the binding energy against particular antigens according to their quality of selfness
311 and dangerousness. The Generalized Quantitative Model of antibody homeostasis proposes that
312 antigen concentrations in the host are adjusted by setting the equilibrium dissociation constants
313 as

$$314 \quad [Ag]=K_D$$

315 Substituting into eq 2),

$$316 \quad [Ag]=\exp(\Delta G^\circ/RT)$$

317 There is an exponential relationship between the adjusted free antigen concentration [Ag] and
318 the binding free energy. The combination of Gaussian distributed interactions of random
319 structures with exponentially distributed antibody frequencies yields a lognormal distribution
320 of free energy levels (Fig.4).

321 Consequently, a normal distribution with mean interaction energy $\mu=0$ and variance $\sigma^2=RT^2$ is
322 transformed into a lognormal distribution with mean $=\exp(\mu + \sigma^2/2)$. This approach gives a
323 mean energy of 27.67 kJ/mol, which is equivalent to $2 \times 10^{-5} K_D$. This is in a good agreement
324 with the observed low affinity antibody binding energy and is also the lower limit of BCR
325 sensitivity (55).

326 In order to define the behavior of a system of molecules we can introduce a system equilibrium
327 constant

$$328 \quad K_{\text{sys}} = K_A / \langle K_A \rangle = e^{-(\Delta G^\circ - \langle \Delta G^\circ \rangle) / RT} \quad 4)$$

329 where $\langle \rangle$ enclose median equilibrium constant and mean free energy of binding, respectively.

330 K_{sys} represents the binding propensity of a given molecule in the system (Fig.3B). The reason
331 for introducing this parameter is that whereas in a bimolecular interaction K_A is a sufficient
332 measure for determining the ratio of bound and free molecules in equilibrium, in a system a
333 given molecule can interact with any other component of the system and its free energy will
334 determine its behavior in the system. Instead of looking at particular bimolecular interactions
335 we are interested in the flow of antigen arising from the sequential interaction with antibodies
336 with increasing affinity.

337 To further explore the properties of the system we shall consider the combination of a
338 lognormal distribution and an exponential distribution by exponential sampling, as has been
339 described by Reed and Mitzenmacher(1,2). The normal distribution of interaction free energy
340 is skewed by each antigen, as explained above, producing lognormal distributions. For the

341 whole system we need to consider these exponentially distributed number of lognormal
342 distributions, generated by the interaction of the system with antigens of exponentially
343 distributed concentrations. The probability density function of K_{sys} with such distribution is
344 given by
345 equation 5)

$$346 \quad f(K_{sys}) = \int_{k=0}^{\infty} \varphi e^{-\varphi k} \frac{1}{\sqrt{2\pi kRTK_{sys}}} e^{-\frac{(\Delta G^{\circ} - k < \Delta G^{\circ} >)^2}{2kRT^2}} dk$$

347
348 using a φ rate of differentiation of cells at every level of differentiation, in other words
349 generation of diversity at rate φ . For the obtained double-Pareto distribution of K_{sys} ,
350 for $K_{sys} > 1$ $f(K_{sys}) = \varphi^2 / 2 \varphi * K_{sys}^{-1-\varphi}$

351 which is a power law distribution of system affinity constants with degree exponent $\varphi - 1 = \varphi^2$.
352 (Fig.4 and 5).

353 We have now obtained the physical chemical description of a system of antibody interactions.

354 *4.2 A scale-free network of interactions*

355 The systematic organization of binding events, as outlined above, can be interpreted as a
356 network of interactions. While antibodies seek to minimize their free energy by finding their
357 best fitting target, antigens are passed on from antibody to antibody. Such shared consecutive
358 binding represents links in the network between nodes of antibodies. Antibodies with the
359 highest K_{sys} value are the most avid binders and as such will take over and handle most antigens,
360 which are channeled to these molecules by antibodies underneath in the binding hierarchy.
361 These antibodies will have the highest number of links and therefore the highest network degree
362 k . The RADARS model in combination with the GQM suggests that long-lived plasma cells
363 act as network hubs. By constantly secreting antibodies, these terminally differentiated cells
364 provide for the binding and removal of antigen of all network nodes, represented by memory
365 B cells, below in the hierarchy. This prevents activation of memory B cells and maintains their
366 resting state. At the bottom of the hierarchy B1 cells, producing natural antibodies, serve as a
367 first line of defense and relay agents for antigen (Fig.6). The higher the network degree and
368 corresponding K_{sys} , the more cells and structure space is covered by a plasma cell.

369 Assuming that $k = K_{sys}$ we obtain the probability density function of the antibody network

$$370 \quad p(k) \sim k^{-\gamma} \quad 6)$$

371 where γ is the degree exponent and is equal to φ^2 (Fig.5).

372 Both cells and secreted antibodies are continually replaced as they die or are removed along
373 with bound target antigens. This in turn regulates the frequency distribution of antibodies along
374 our radius of $\log K_{sys}$.

375 The emerging power law relationship of antibody interactions is a hallmark of scale-free
376 networks (57). This scale-free network is an energy transfer system physically and an antigen
377 transfer system immunologically. This is an optimization of antibody differentiation in the
378 sense that the minimal number of high free energy antibodies (network hubs) are used for the
379 removal of the maximal number of antigens, covering the maximum of immunologically
380 relevant structure space. The generation of an antibody network, with network hubs represented
381 by plasma cells secreting antibodies, reveals the physical aspect of the system: all interactions
382 of such an antibody contribute to the clearance of many target antigens sharing structural
383 homology. A new node in the network, a new B cell in the structure space, will preferentially
384 attach to an existing subnetwork as a low-affinity clone, in agreement with the preferential
385 attachment model of growth in scale-free networks (58). Preferential attachment may explain
386 immunodominance and antigenic sin, phenomena arising from the preference of the immune
387 system for known epitopes, which correspond to hubs in the network.

388 *4.3 An inverted view of the system*

389 The model presented so far follows a view of the system growing outwards, with cells supplied
390 from within and extending into the world of antigens. However, we can turn this view inside
391 out, as shown in Figure 5, interpreting the events as the immune system being outside and
392 developing inwards. Practically this means that we use the dissociation constant K_D instead of
393 K_A as a measure for the system. Though it is more difficult to visualize a system that grows
394 inwards, this view helps our perception of the developing antibody network and of antigen
395 removal.

396 In the immune-side out view of the RADARS model distance from the outside boundary of the
397 system represents resolution of molecular recognition, which is now K_D . Directions still
398 correspond to theoretical targets: individual molecular shapes. If all these targets have binding
399 energy distributions as predicted by the universal distribution model, then our interaction space
400 will represent a collection of these statistical distributions.

401 The RADARS model suggests that the greater the resolution of structural recognition the more
402 restricted is the number of shapes recognized. However, with the development of high affinity
403 clones the ability to react with related structures also grows, a phenomenon called cross
404 reactivity. The further the system grows in a given direction the more focused is the recognition
405 of cognate target but affinity to related structures inevitably grows as well. This is not mere
406 polyreactivity, however, but rather organized cross-reactivity. With the supply of B-cell
407 precursors outside and the organization of antigen removal inside, we can best interpret effector
408 antibody function as an antigen sink (Fig.7). In this sink multiple sinkholes develop as the

409 immune system matures. The sinkholes themselves correspond to immunodominant epitopes:
410 structures preferred by the system as targets.

411

412 **5. Merging sequence space to interaction space**

413

414 Network theory has always been considered as a key to understand and define immunity on a
415 systems level. The network hypothesis of Niels Jerne (23), its modified version leading to the
416 concept of clonal selection (59), mathematical and computational simulations (60,61), various
417 re-interpretations (24), experimental approaches using NGS (25,31) or antigen microarrays
418 (22) all strive to describe this highly complex system as connected elements of a network.
419 There are two new aspects of the RADARS model that may improve our view of this network.
420 First, it introduces physical units, binding energy, as the measure of interactions and as a
421 measure of system architecture. Natural networks are formed as a result of energy dispersal
422 (62,63), therefore network theories should consider energy transduction in the system. Second,
423 it proposes an architecture for the whole network, characterized by the scale-free distribution,
424 and an optimal value for the degree exponent of power-law relationship.

425 The network architecture of antibody repertoires was recently computed based on high-
426 throughput sequencing data from more than 100.000 unique antibody sequences (64). This
427 study revealed that pre-B cell and naïve B-cell clones form homogenously interconnected
428 assortative networks, in contrast to the disassortative networks of plasma cell clones, which
429 covered smaller but more focused regions of sequence space. This contrasting behavior of
430 antigen-naïve and antigen experienced, post-germinal center B cells corresponds to the
431 antibody-centric view in our model. The low-affinity region with developing B-cells is
432 homogenously interconnected by clonal relationships and shared usage of gene segments (Fig.2
433 and 3). The high affinity side of the distribution is the narrowing, focusing interaction space of
434 plasma cells.

435 Considering that our technological capability is ripe for the high-resolution determination and
436 comprehensive analysis of antibody sequence space, current efforts focus on the conversion of
437 sequence space data into datasets in interaction space. By providing a physical and
438 mathematical description of relationship between antibody clones the RADARS model may
439 help in the final integration of sequence data. The model also suggests that sequence-based
440 network properties of early B-cell developmental stages also need to be determined (25), in
441 addition to the mature and antigen-experienced repertoire (65), and comprehensive and

442 selective analysis of the B1 repertoire is very important for capturing network properties of the
443 system.

444 The model presented here with a network degree exponent ϕ^2 depicts an ideal state of the
445 system of antibody interactions. It is the fluctuations and disturbances in the system that we
446 observe as immune response during infections, and distortions are autoimmunity and allergy.
447 Besides suggesting how antibody sequence space fits into structural space and into an
448 interaction network, the model may potentially lead to the ability to model whole immune
449 system and simulate its functioning.

450

451 **6. Concluding remarks**

452 This theoretical study introduces the concept of antibody interaction space, which arises from
453 structure space, and is based on the distribution of interaction energy. In a physical chemistry
454 perspective, the immune system is a grand canonical ensemble: the number of states with given
455 energy corresponds to conformational diversity of antibodies, the probability of states is
456 dictated by the availability of antigen, the host serves as a heat bath and particle bath,
457 maintaining temperature and being a source of antigen, finally, chemical potential is kept the
458 same by immune mechanisms adjusting antibody concentration according to K_D . A self-
459 organizing system, such as the humoral adaptive immune system, is based on the organization
460 of interactions. Since molecular interactions are determined by structure, organized interaction
461 space should mean an organized structure space. The RADARS model proposes that a
462 universal organization of an immense number of structures in a huge but finite system is
463 possible by adjusting the resolution of structural recognition, which is the adjustment of
464 interaction energy. Radial adjustment of system resolution generates a network of interactions.
465 Consecutive binding reactions generate a stationary non-equilibrium system. The network of
466 interactions is scale-free and is characterized by a power law distribution of free energy of
467 interactions. Overall, this organization allows the energy optimized controlled removal of
468 antigens from the host system.

469

470 Acknowledgments

471 The paper is dedicated to my first mentor in molecular biology and antibody cloning, István
472 Kurucz. The author is partly supported by VEKOP 2.1.1-15-00098 grant to Diagnosticum. I
473 wish to thank István Csabai for reading and discussing the manuscript and anonymous referees
474 for their constructive criticism. Special thanks to Tamás Pfeil and Ágnes Kovács for
475 enlightening discussions about exponential distributions.

476

477 Author contributions

478 J.P. developed theoretical model, designed and prepared figures, wrote paper

479

480 Conflicts of interest

481 The author declares no conflict of interest.

482

483

484 References

- 485 1. Müller V, de Boer RJ, Bonhoeffer S, Szathmáry E. An evolutionary perspective on the
486 systems of adaptive immunity. *Biol Rev Camb Philos Soc.* 2017 Jul 26;93(1):505–528.
- 487 2. Eberl G. A new vision of immunity: homeostasis of the superorganism. *Mucosal*
488 *Immunol.* 2010 Sep;3(5):450–460.
- 489 3. Sleator RD. The human superorganism - of microbes and men. *Med Hypotheses.* 2010
490 Feb;74(2):214–215.
- 491 4. Pradeu T, Carosella ED. On the definition of a criterion of immunogenicity. *Proc Natl*
492 *Acad Sci U S A.* 2006 Nov 21;103(47):17858–17861.
- 493 5. Davis MM, Tato CM, Furman D. Systems immunology: just getting started. *Nat*
494 *Immunol.* 2017 Jun 20;18(7):725–732.
- 495 6. Prechl J. A generalized quantitative antibody homeostasis model: maintenance of
496 global antibody equilibrium by effector functions. *Clin Transl Immunology.* 2017 Nov
497 17;6(11):e161.
- 498 7. Boyd SD, Joshi SA. High-Throughput DNA Sequencing Analysis of Antibody
499 Repertoires. *Microbiol Spectr.* 2014 Oct;2(5).
- 500 8. Vergani S, Korsunsky I, Mazzarello AN, Ferrer G, Chiorazzi N, Bagnara D. Novel
501 Method for High-Throughput Full-Length IGHV-D-J Sequencing of the Immune
502 Repertoire from Bulk B-Cells with Single-Cell Resolution. *Front Immunol.* 2017 Sep
503 14;8:1157.
- 504 9. Friedensohn S, Lindner JM, Cornacchione V, Iazeolla M, Miho E, Zingg A, et al.
505 Synthetic standards combined with error and bias correction improve the accuracy and
506 quantitative resolution of antibody repertoire sequencing in human naïve and memory
507 B cells. *Front Immunol.* 2018 Jun 20;9:1401.
- 508 10. Miho E, Yermanos A, Weber CR, Berger CT, Reddy ST, Greiff V. Computational
509 Strategies for Dissecting the High-Dimensional Complexity of Adaptive Immune
510 Repertoires. *Front Immunol.* 2018 Feb 21;9:224.
- 511 11. Greiff V, Miho E, Menzel U, Reddy ST. Bioinformatic and statistical analysis of
512 adaptive immune repertoires. *Trends Immunol.* 2015 Nov;36(11):738–749.
- 513 12. Prechl J, Papp K, Erdei A. Antigen microarrays: descriptive chemistry or functional
514 immunomics? *Trends Immunol.* 2010 Apr;31(4):133–137.
- 515 13. Merbl Y, Zucker-Toledano M, Quintana FJ, Cohen IR. Newborn humans manifest
516 autoantibodies to defined self molecules detected by antigen microarray informatics. *J*
517 *Clin Invest.* 2007 Mar;117(3):712–718.
- 518 14. Bacarese-Hamilton T, Bistoni F, Crisanti A. Protein microarrays: from serodiagnosis to
519 whole proteome scale analysis of the immune response against pathogenic
520 microorganisms. *BioTechniques.* 2002 Dec;Suppl:24–29.
- 521 15. Wang J, Lin J, Bardina L, Goldis M, Nowak-Wegrzyn A, Shreffler WG, et al.
522 Correlation of IgE/IgG4 milk epitopes and affinity of milk-specific IgE antibodies with
523 different phenotypes of clinical milk allergy. *J Allergy Clin Immunol.* 2010
524 Mar;125(3):695–702, 702.e1.
- 525 16. Grötzinger C. Peptide microarrays for medical applications in autoimmunity, infection,
526 and cancer. *Methods Mol Biol.* 2016;1352:213–221.

- 527 17. Sjöberg R, Mattsson C, Andersson E, Hellström C, Uhlen M, Schwenk JM, et al.
528 Exploration of high-density protein microarrays for antibody validation and
529 autoimmunity profiling. *N Biotechnol.* 2016 Sep 25;33(5 Pt A):582–592.
- 530 18. Bradford TJ, Wang X, Chinnaiyan AM. Cancer immunomics: using autoantibody
531 signatures in the early detection of prostate cancer. *Urol Oncol.* 2006 Jun;24(3):237–
532 242.
- 533 19. Caetano-Anollés G, Wang M, Caetano-Anollés D, Mittenthal JE. The origin, evolution
534 and structure of the protein world. *Biochem J.* 2009 Feb 1;417(3):621–637.
- 535 20. Scaviner D, Barbié V, Ruiz M, Lefranc MP. Protein displays of the human
536 immunoglobulin heavy, kappa and lambda variable and joining regions. *Exp Clin*
537 *Immunogenet.* 1999;16(4):234–240.
- 538 21. Krawczyk K, Kelm S, Kovaltsuk A, Galson JD, Kelly D, Trück J, et al. Structurally
539 mapping antibody repertoires. *Front Immunol.* 2018 Jul 23;9:1698.
- 540 22. Madi A, Kenett DY, Bransburg-Zabary S, Merbl Y, Quintana FJ, Tauber AI, et al.
541 Network theory analysis of antibody-antigen reactivity data: the immune trees at birth
542 and adulthood. *PLoS ONE.* 2011 Mar 8;6(3):e17445.
- 543 23. Jerne NK. Towards a network theory of the immune system. *Ann Immunol (Paris).*
544 1974 Jan;125C(1-2):373–389.
- 545 24. Coutinho A. The network theory: 21 years later. *Scand J Immunol.* 1995 Jul;42(1):3–8.
- 546 25. Miho E, Greiff V, Roskar R, Reddy ST. The fundamental principles of antibody
547 repertoire architecture revealed by large-scale network analysis. *BioRxiv.* 2017 Apr 5;
- 548 26. Prechl J. A generalized quantitative antibody homeostasis model: regulation of B-cell
549 development by BCR saturation and novel insights into bone marrow function. *Clin*
550 *Transl Immunology.* 2017 Feb 17;6(2):e130.
- 551 27. Prechl J. A generalized quantitative antibody homeostasis model: antigen saturation,
552 natural antibodies and a quantitative antibody network. *Clin Transl Immunology.* 2017
553 Feb 24;6(2):e131.
- 554 28. Elhanati Y, Sethna Z, Marcou Q, Callan CG, Mora T, Walczak AM. Inferring processes
555 underlying B-cell repertoire diversity. *Philos Trans R Soc Lond, B, Biol Sci.* 2015 Sep
556 5;370(1676).
- 557 29. Kovaltsuk A, Leem J, Kelm S, Snowden J, Deane CM, Krawczyk K. Observed
558 Antibody Space: A Resource for Data Mining Next-Generation Sequencing of
559 Antibody Repertoires. *J Immunol.* 2018 Sep 14;
- 560 30. Rettig TA, Ward C, Bye BA, Pecaut MJ, Chapes SK. Characterization of the naive
561 murine antibody repertoire using unamplified high-throughput sequencing. *PLoS ONE.*
562 2018 Jan 10;13(1):e0190982.
- 563 31. Bashford-Rogers RJM, Palser AL, Huntly BJ, Rance R, Vassiliou GS, Follows GA, et
564 al. Network properties derived from deep sequencing of human B-cell receptor
565 repertoires delineate B-cell populations. *Genome Res.* 2013 Nov;23(11):1874–1884.
- 566 32. D’Angelo S, Ferrara F, Naranjo L, Erasmus MF, Hraber P, Bradbury ARM. Many
567 Routes to an Antibody Heavy-Chain CDR3: Necessary, Yet Insufficient, for Specific
568 Binding. *Front Immunol.* 2018 Mar 8;9:395.

- 569 33. Greiff V, Menzel U, Miho E, Weber C, Riedel R, Cook S, et al. Systems Analysis
570 Reveals High Genetic and Antigen-Driven Predetermination of Antibody Repertoires
571 throughout B Cell Development. *Cell Rep.* 2017 May 16;19(7):1467–1478.
- 572 34. DeFalco J, Harbell M, Manning-Bog A, Baia G, Scholz A, Millare B, et al. Non-
573 progressing cancer patients have persistent B cell responses expressing shared antibody
574 paratopes that target public tumor antigens. *Clin Immunol.* 2018;187:37–45.
- 575 35. Bukhari SAC, O'Connor MJ, Martínez-Romero M, Egyedi AL, Willrett D, Graybeal J,
576 et al. The CAIRR Pipeline for Submitting Standards-Compliant B and T Cell Receptor
577 Repertoire Sequencing Studies to the National Center for Biotechnology Information
578 Repositories. *Front Immunol.* 2018 Aug 16;9:1877.
- 579 36. Breden F, Luning Prak ET, Peters B, Rubelt F, Schramm CA, Busse CE, et al.
580 Reproducibility and reuse of adaptive immune receptor repertoire data. *Front Immunol.*
581 2017 Nov 1;8:1418.
- 582 37. Rubelt F, Busse CE, Bukhari SAC, Bürckert J-P, Mariotti-Ferrandiz E, Cowell LG, et
583 al. Adaptive Immune Receptor Repertoire Community recommendations for sharing
584 immune-repertoire sequencing data. *Nat Immunol.* 2017 Nov 16;18(12):1274–1278.
- 585 38. Rosenfeld AM, Meng W, Luning Prak ET, Hershberg U. Immunedb, a novel tool for
586 the analysis, storage, and dissemination of immune repertoire sequencing data. *Front*
587 *Immunol.* 2018 Sep 21;9:2107.
- 588 39. Corrie BD, Marthandan N, Zimonja B, Jaglale J, Zhou Y, Barr E, et al. iReceptor: A
589 platform for querying and analyzing antibody/B-cell and T-cell receptor repertoire data
590 across federated repositories. *Immunol Rev.* 2018;284(1):24–41.
- 591 40. Avram O, Vaisman-Mentesh A, Yehezkel D, Ashkenazy H, Pupko T, Wine Y. ASAP -
592 A Webserver for Immunoglobulin-Sequencing Analysis Pipeline. *Front Immunol.* 2018
593 Jul 30;9:1686.
- 594 41. Kringelum JV, Nielsen M, Padkjær SB, Lund O. Structural analysis of B-cell epitopes
595 in antibody:protein complexes. *Mol Immunol.* 2013 Jan;53(1-2):24–34.
- 596 42. Wedemayer GJ, Patten PA, Wang LH, Schultz PG, Stevens RC. Structural insights into
597 the evolution of an antibody combining site. *Science.* 1997 Jun 13;276(5319):1665–
598 1669.
- 599 43. Chen J, Sawyer N, Regan L. Protein-protein interactions: general trends in the
600 relationship between binding affinity and interfacial buried surface area. *Protein Sci.*
601 2013 Apr;22(4):510–515.
- 602 44. Brooijmans N, Sharp KA, Kuntz ID. Stability of macromolecular complexes. *Proteins.*
603 2002 Sep 1;48(4):645–653.
- 604 45. Melchers F. Checkpoints that control B cell development. *J Clin Invest.* 2015
605 Jun;125(6):2203–2210.
- 606 46. Prechl J. Thermodynamic projection of the antibody interaction network: the fountain
607 energy landscape of binding. *BioRxiv.* 2017 Apr 5;
- 608 47. Derényi I, Szöllösi GJ. Hierarchical tissue organization as a general mechanism to limit
609 the accumulation of somatic mutations. *Nat Commun.* 2017 Feb 23;8:14545.
- 610 48. Cohen IR. Biomarkers, self-antigens and the immunological homunculus. *J*
611 *Autoimmun.* 2007 Dec;29(4):246–249.

- 612 49. Tangye SG, Tarlinton DM. Memory B cells: effectors of long-lived immune responses.
613 Eur J Immunol. 2009 Aug;39(8):2065–2075.
- 614 50. Shah HB, Smith K, Wren JD, Webb CF, Ballard JD, Bourn RL, et al. Insights From
615 Analysis of Human Antigen-Specific Memory B Cell Repertoires. Front Immunol.
616 2018;9:3064.
- 617 51. Neuberger MS, Ehrenstein MR, Rada C, Sale J, Batista FD, Williams G, et al. Memory
618 in the B-cell compartment: antibody affinity maturation. Philos Trans R Soc Lond, B,
619 Biol Sci. 2000 Mar 29;355(1395):357–360.
- 620 52. Good-Jacobson KL, Shlomchik MJ. Plasticity and heterogeneity in the generation of
621 memory B cells and long-lived plasma cells: the influence of germinal center
622 interactions and dynamics. J Immunol. 2010 Sep 15;185(6):3117–3125.
- 623 53. Hammarlund E, Thomas A, Amanna IJ, Holden LA, Slayden OD, Park B, et al. Plasma
624 cell survival in the absence of B cell memory. Nat Commun. 2017 Nov 24;8(1):1781.
- 625 54. Zheng X, Wang J. The universal statistical distributions of the affinity, equilibrium
626 constants, kinetics and specificity in biomolecular recognition. PLoS Comput Biol.
627 2015 Apr 17;11(4):e1004212.
- 628 55. Batista FD, Neuberger MS. Affinity dependence of the B cell response to antigen: a
629 threshold, a ceiling, and the importance of off-rate. Immunity. 1998 Jun;8(6):751–759.
- 630 56. Dorogovtsev SN (Sergei N.), Mendes JFF (José FF). Evolution of networks: From
631 biological nets to the Internet and WWW. Paperback. Oxford: Oxford University Press;
632 2003.
- 633 57. Albert R, Barabási A-L. Statistical mechanics of complex networks. Rev Mod Phys.
634 2002 Jan 30;74(1):47–97.
- 635 58. Barabási A-L, Oltvai ZN. Network biology: understanding the cell's functional
636 organization. Nat Rev Genet. 2004 Feb;5(2):101–113.
- 637 59. Burnet FM. A modification of Jerne's theory of antibody production using the concept
638 of clonal selection. CA Cancer J Clin. 1976 Apr;26(2):119–121.
- 639 60. FARMER DJ, PACKARD N, PERELSON A. The immune system, adaptation, and
640 machine learning. Physica D: Nonlinear Phenomena. 1986;22(1-3):187–204.
- 641 61. Schulz R, Werner B, Behn U. Self-tolerance in a minimal model of the idiotypic
642 network. Front Immunol. 2014 Mar 10;5:86.
- 643 62. Mäkelä T, Annala A. Natural patterns of energy dispersal. Phys Life Rev. 2010
644 Dec;7(4):477–498.
- 645 63. Hartonen T, Annala A. Natural networks as thermodynamic systems. Complexity. 2012
646 Nov;18(2):53–62.
- 647 64. Miho E, Roškar R, Greiff V, Reddy ST. Large-scale network analysis reveals the
648 sequence space architecture of antibody repertoires. Nat Commun. 2019 Mar
649 21;10(1):1321.
- 650 65. Kono N, Sun L, Toh H, Shimizu T, Xue H, Numata O, et al. Deciphering antigen-
651 responding antibody repertoires by using next-generation sequencing and confirming
652 them through antibody-gene synthesis. Biochem Biophys Res Commun. 2017 May
653 27;487(2):300–306.
- 654

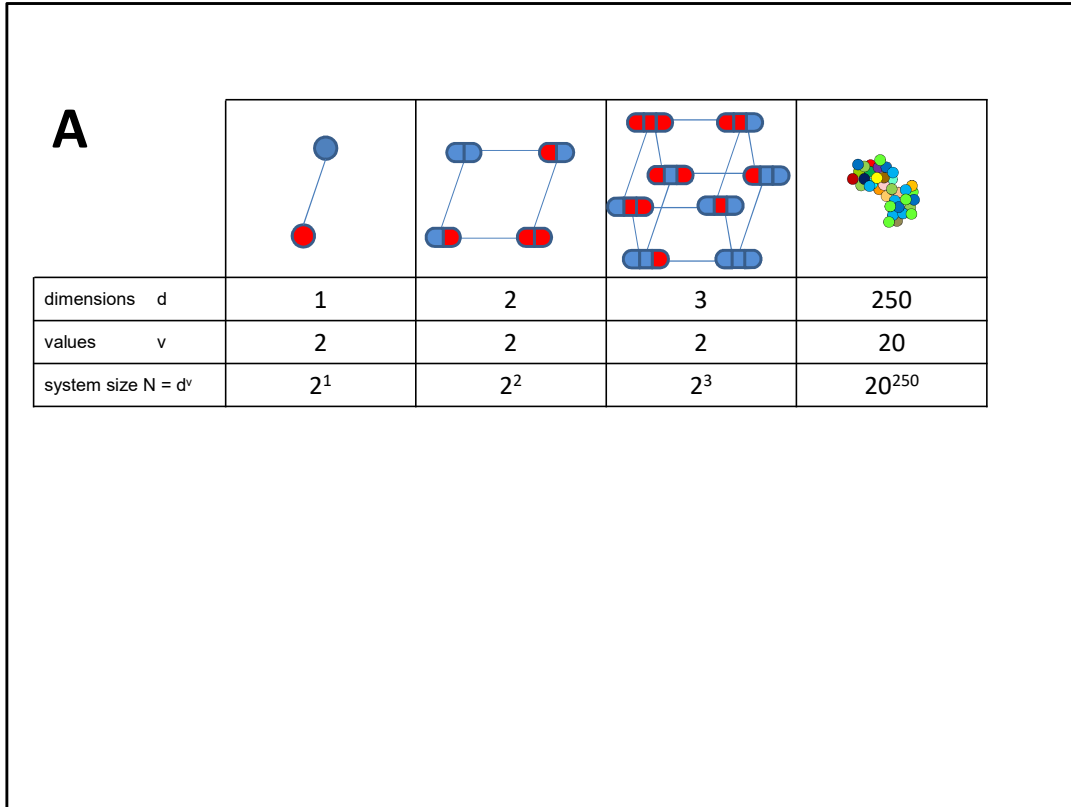


Figure 1. Sequence space and visualization of antibody sequence relationships
A) Theoretical diversity of a sequence is determined by its length and the number of values a particular position in the sequence can take. More than 3 dimensions are difficult to visualize in 2D. An antibody Fv region of 250 amino acids has an astronomical sequence diversity if full randomization is allowed. If the sequences are exact the positions in sequence space are discrete, but there are no structurally meaningful directions or distances in this multidimensional sequence space with N different sequences.

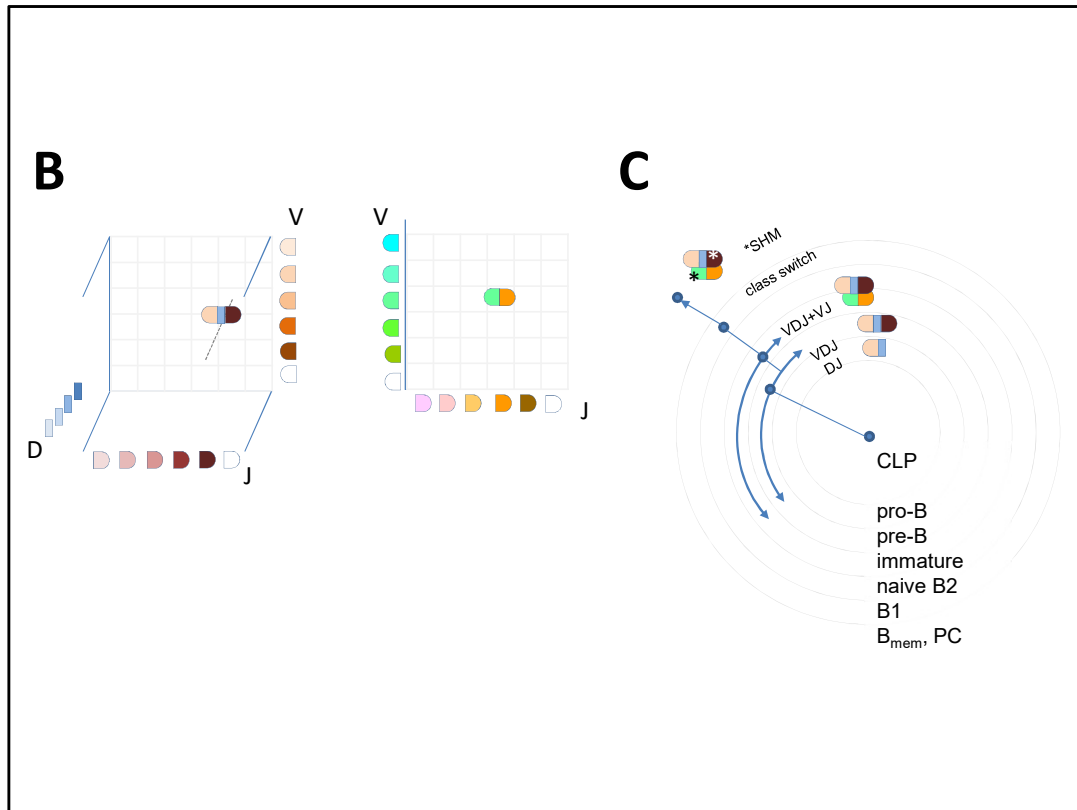


Figure 1

B) Antibody sequences are frequently interpreted as recombined germline sequences. This approach allows the simplified display of repertoires obtained by NGS, preferably with paired heavy and light chain VD identification. Such a display of combinatorial diversity may allow the tracking of specific clonal expansions and further diversification by SHM but reveals little about the overall functional network of interactions.

C) The potential development scheme of a given antibody clone is shown with antibody sequence development along with B-cell differentiation steps. Arching arrows represent combinatorial diversification by V-D-J rearrangement and light chain pairing.

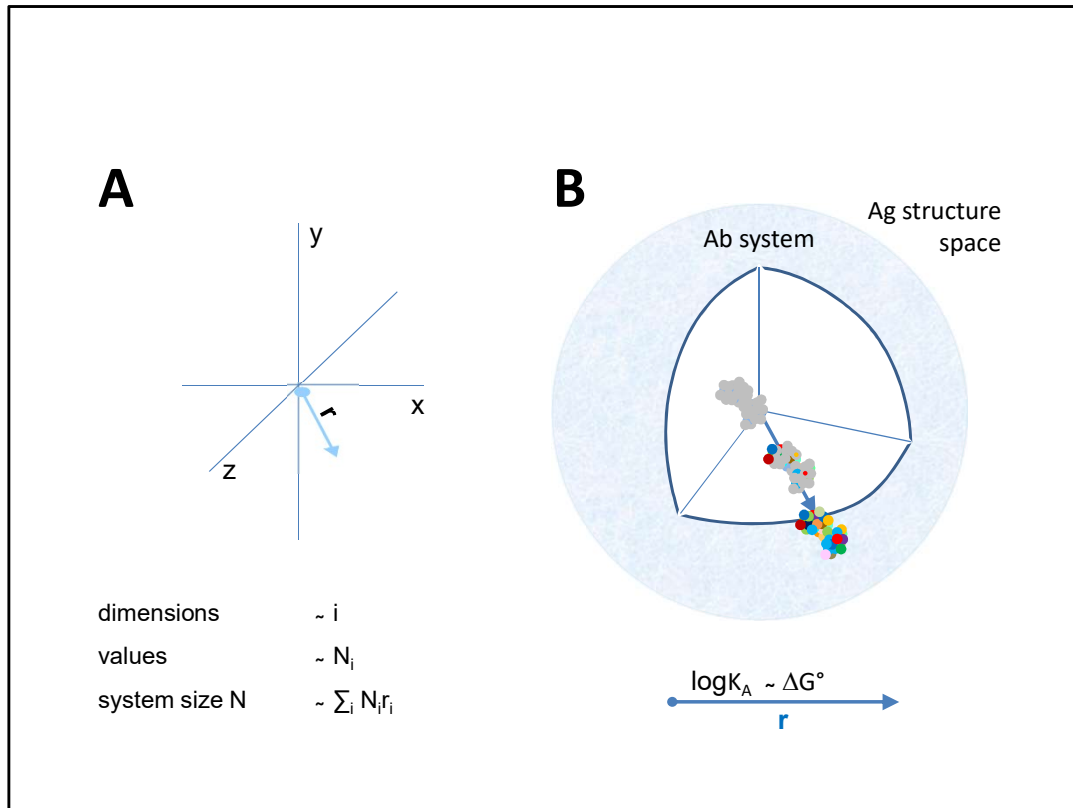


Figure 2. Quantitative interaction space and B-cell differentiation

A) The system of interactions has a center as a reference point in a conceptual three dimensional space. Structural diversity, that is different shapes, appear as directions (exemplary arrow) from this center towards the target structure in Ag structure space. Distinct directions can be defined with a precision dependent on the distance from the center, equivalent to the radius (r) of the system extending in that particular direction. Multidimensionality is theoretically infinite in this representation, practical limits being introduced by the maximum interaction energy (ΔG°) in the system. For i dimensions with radius r_i , each with N_i number of distinct structures, the total size of the system N is the sum of structures of all dimensions.

B) Structural diversity appears as we leave the center, spherical shells representing various levels of resolution of molecular recognition, measured as $\log K_A$. Colors represent distinct amino acids of antibody binding site engaging in non-covalent bonding with the target molecule. As the Ab molecule approaches its native, bound state the number of engaged residues increases.

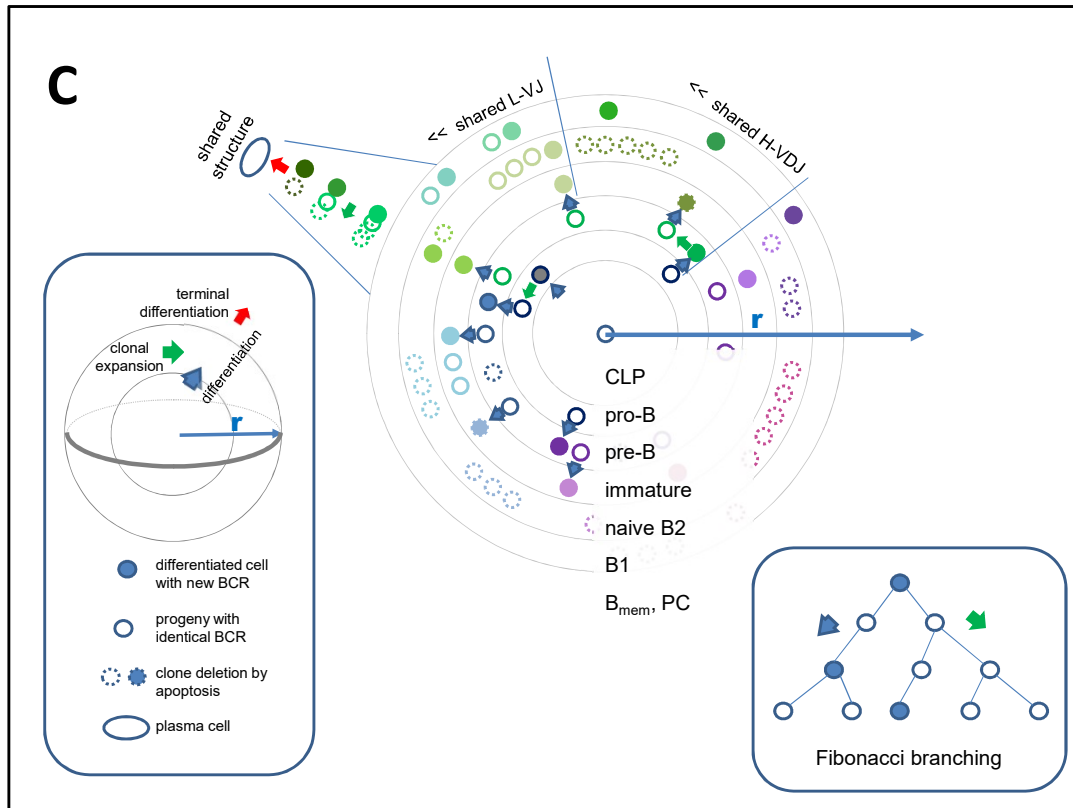


Figure 2.

C) Systemic organization of antibody evolution.

The evolution of the system of antibodies can be interpreted as clones filling the interaction space at various levels of resolution. Along this pathway cells continually increase their specificity and affinity towards their target direction. A common lymphoid progenitor has the potential to develop antibody against any target. Differentiation proceeds outwards from this point of origin. At every level of differentiation asymmetric divisions fill the level with a clone, expansion being dictated by antigen driven selection of clones with ideal receptor engagement. TD responses allow further directed differentiation via somatic hypermutations in germinal centers, yielding post-germinal center B cells, that is memory B cells and plasma cells. Naive B2 cells are continuously generated and only survive if recruited for specific immune responses. B1 cells, on the contrary, survive in an activated state producing antibodies and dividing rapidly upon activation. Fibonacci branching of dividing and differentiating cells defines ratios of cells on various levels. Different colors stand for structural differences and relationships.

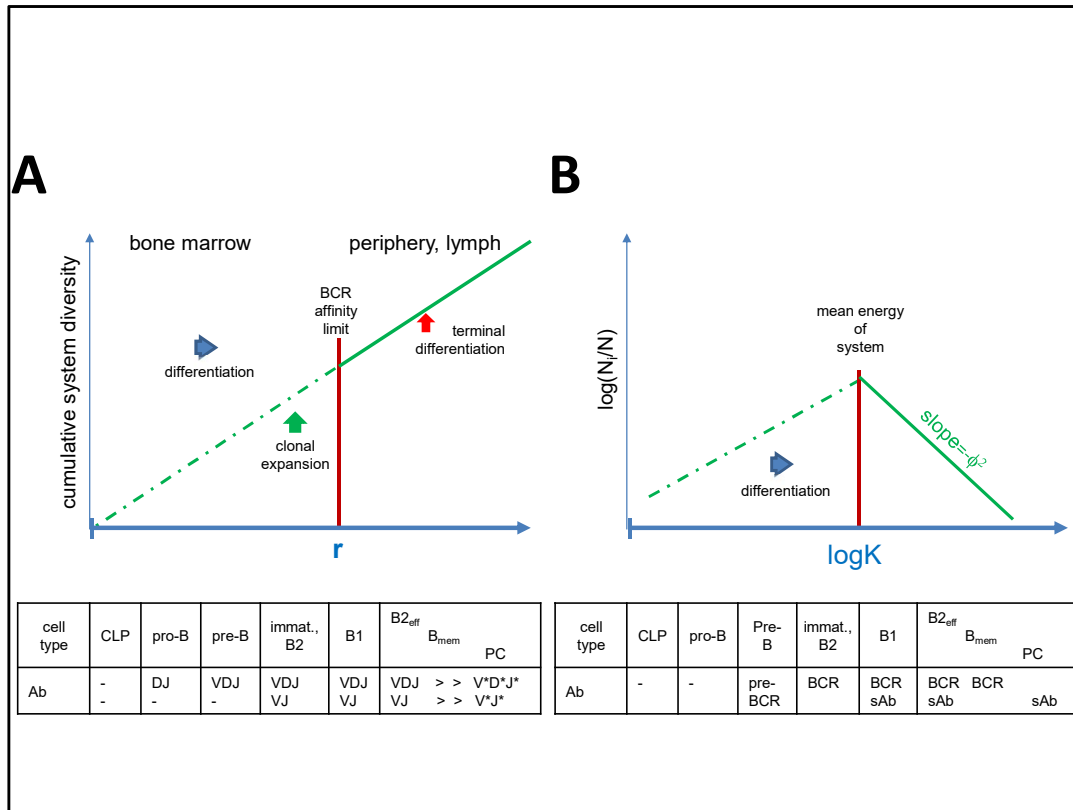


Figure 3. Predicted interaction properties of the humoral immune system.

A) Clonal network development of the humoral immune system, shown as cumulative distribution of evolving antibody clones. The self-organizing system in the bone marrow utilizes recombination of gene segments (V,D,J) and meets environment in the periphery to maintain immunological self and generate immunological antibody profile, here also employing selected somatic mutations (*). r' represents the radius of the system. B) Distribution of equilibrium constant of antibody interactions in the system. *somatic hypermutations; CLP, common lymphoid progenitor; immat., immature; B2eff, effector B2 cell; Bmem, memory B cell; PC, plasma cell; BCR, B-cell receptor; sAb, secreted antibody

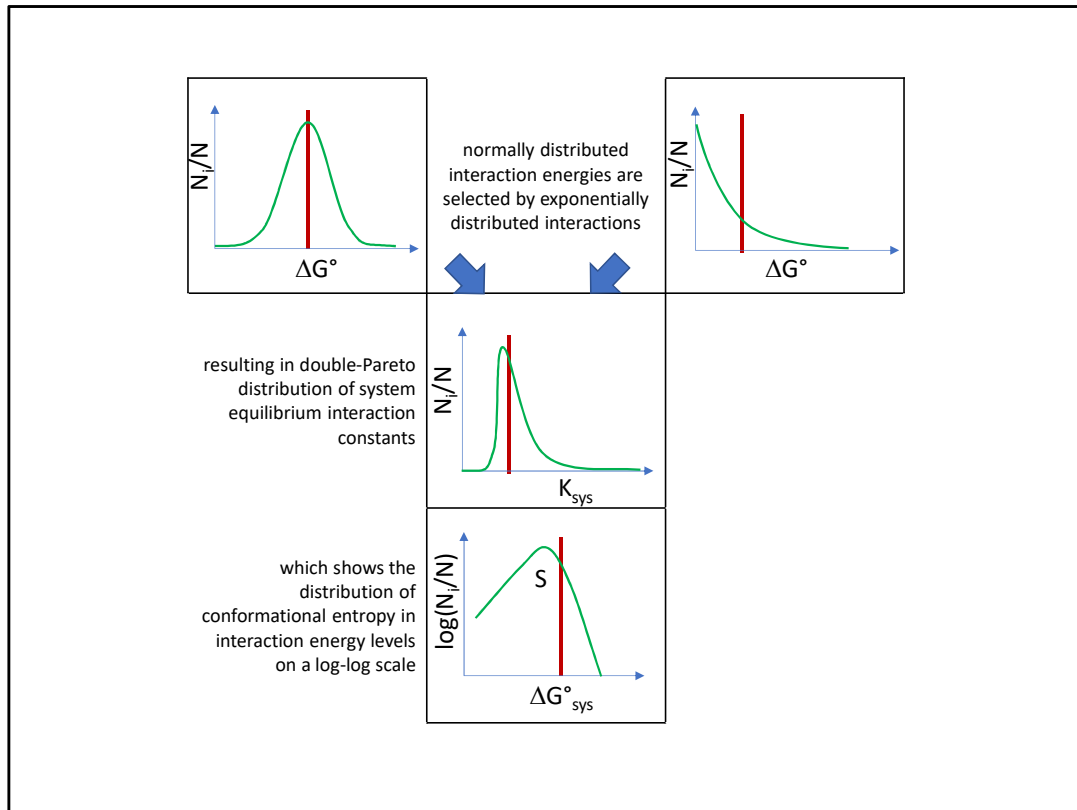


Figure 4. Combination of distributions.
Red lines represent the expected values of the distributions.

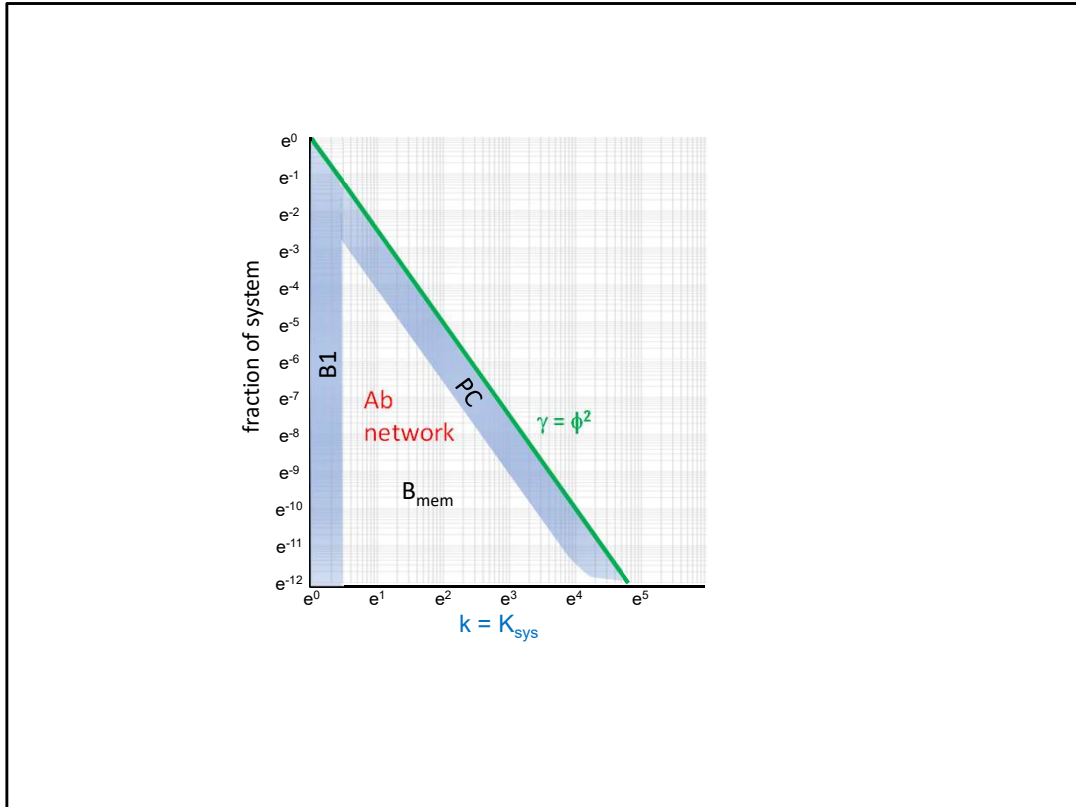


Figure 5. Properties of the antibody interaction network.

Degree distribution of the antibody network corresponds to the probability distribution of relative free energy of antibody interactions, where the system equilibrium constant is the degree of a B-cell clone node and N_i/N is relative frequency of nodes with that degree in the interaction network. Note that this figure corresponds to the lower right quadrant of Figure 3B, except that not the axis values but the scale is logarithmic. B1 cells have the lowest degree, plasma cells have the highest energy at a particular frequency, memory B cells form the rest of the nodes.

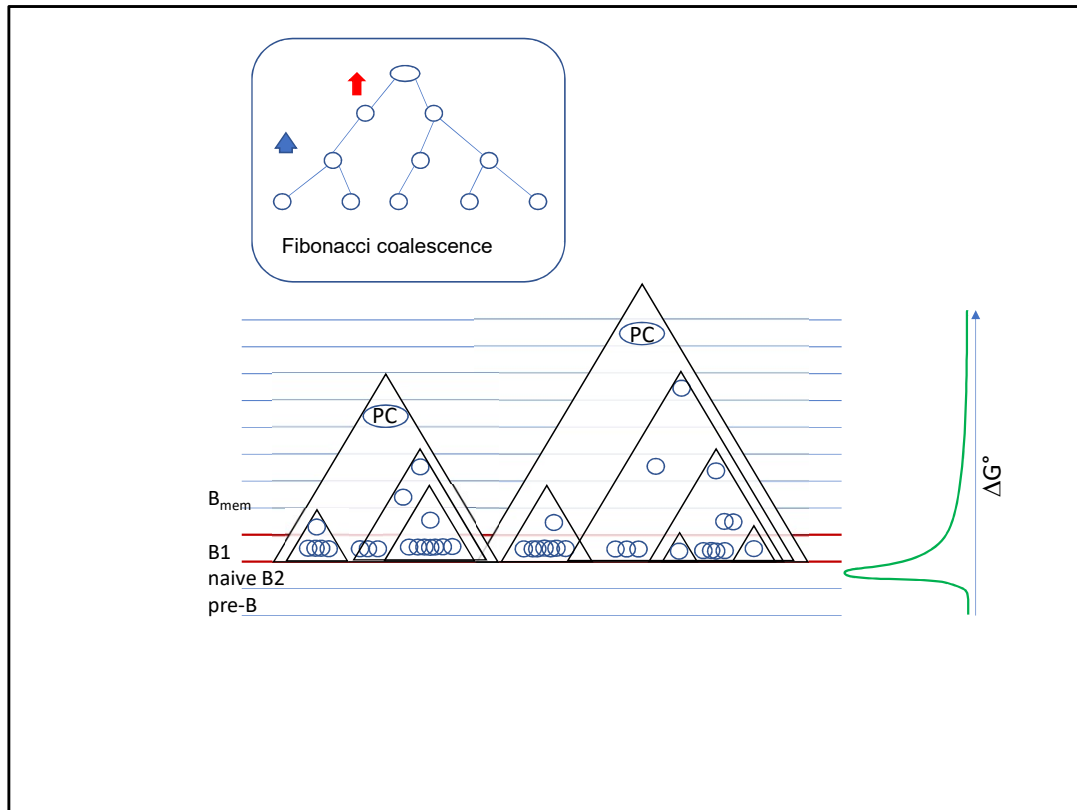


Figure 6. Network organization of antibody responses

Assuming that the rate of coalescence of structural diversity is identical to the diversification, on a systemic scale it will be phi. In subnetworks plasma cells act as hubs, being connected to all memory B cells and B1 cells with shared structures at lower free energy levels. By secreting antibodies, plasma cells control activation of all connected cells. Triangles represent subnetworks, blue lines indicate energy levels.

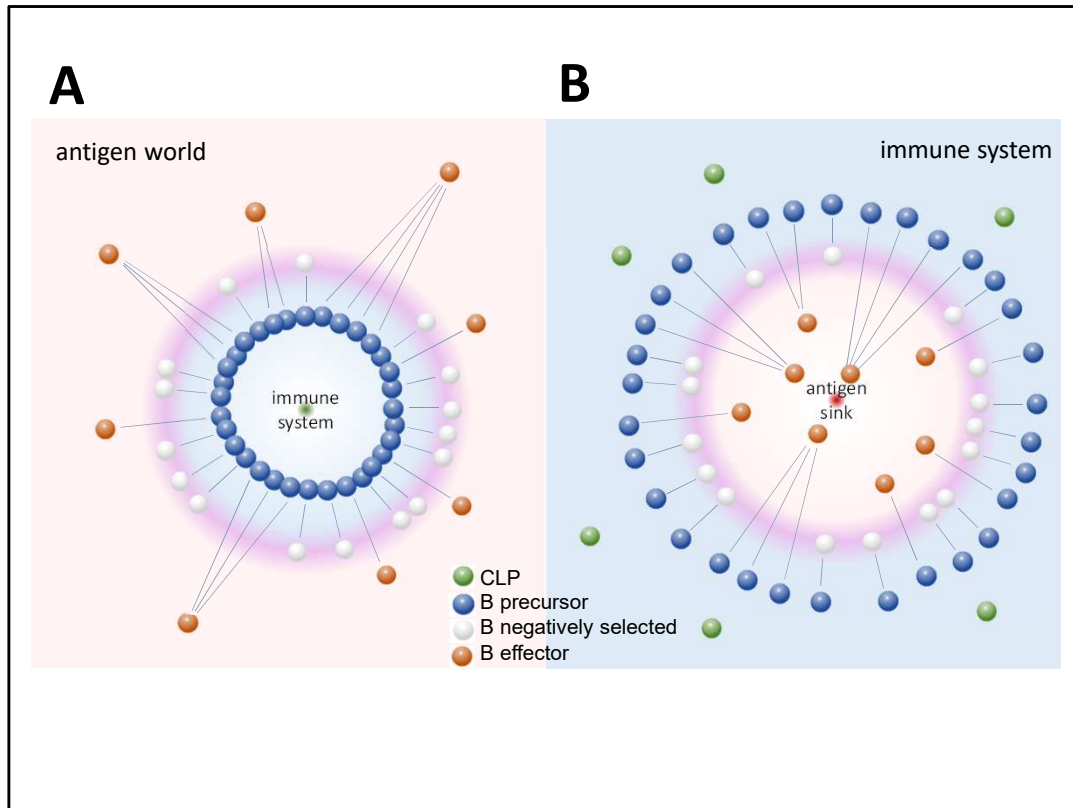
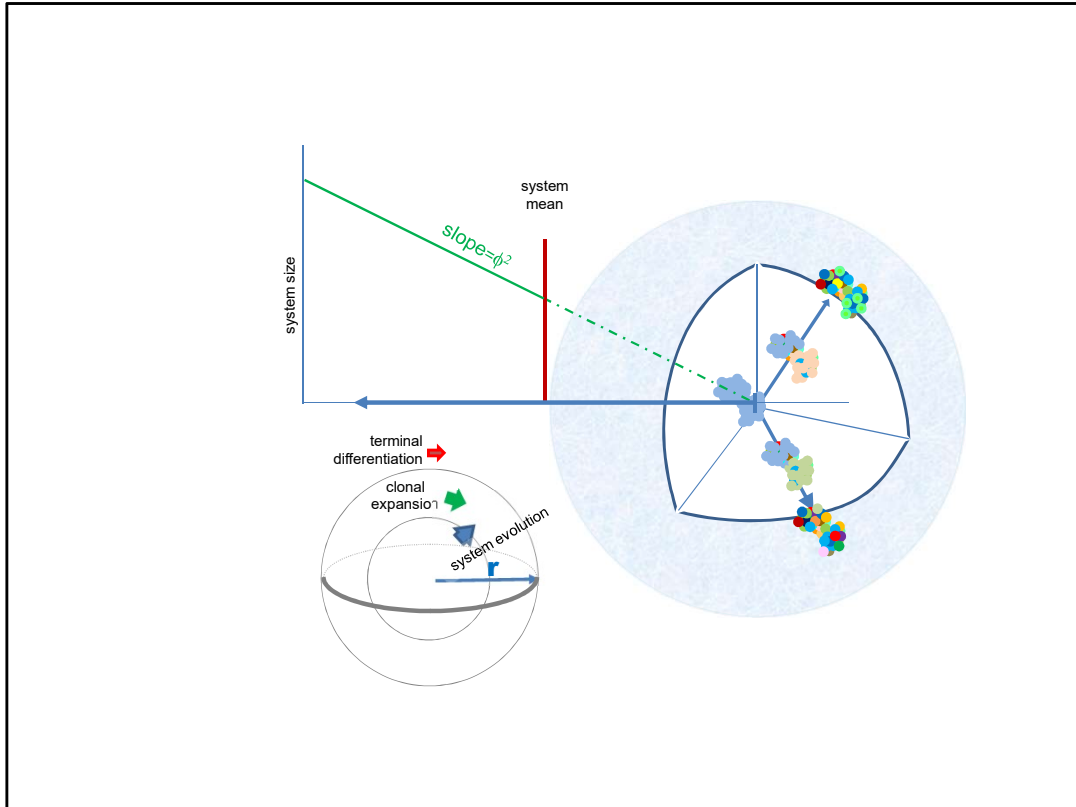


Figure 7. Antibody-centric and antigen-centric views of the organization of adaptive humoral immunity.

The antibody-centric view (A) corresponds to the clonal development and expansion of B cells, generating and maintaining a spherical system with a radius of $\ln K_{\text{sys}}$. The system carves out a niche in the molecular structural world of antigens. We can turn the system inside out to obtain an antigen-centric view (B), where the system grows „inwards” with a radius corresponding to $\ln(1/K_{\text{sys}})$. In this representation the development of antibodies with higher affinity pull antigen down an antigen sink with increasing efficiency, capturing antigen of a wider structural diversity.



Graphical abstract